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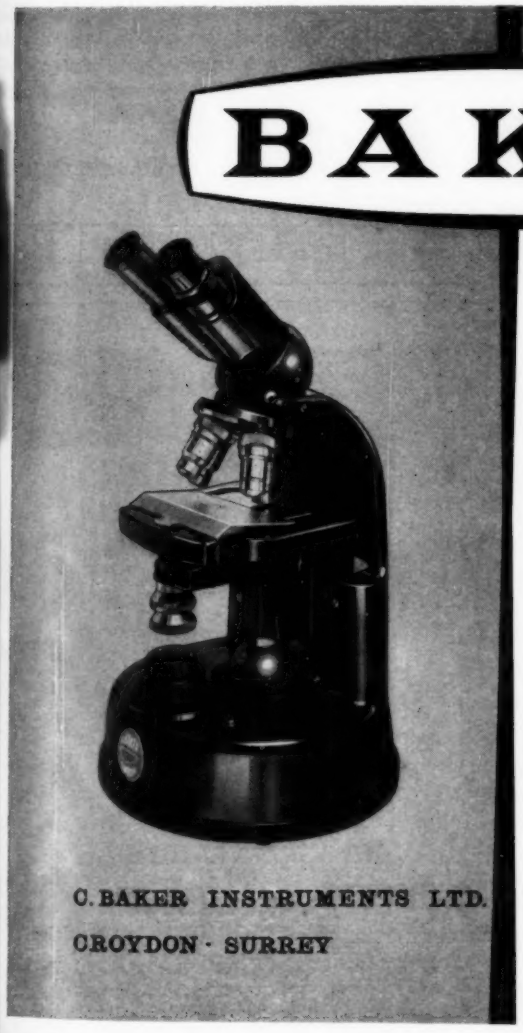

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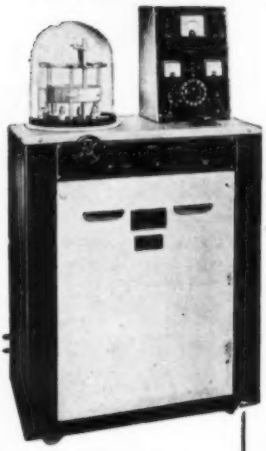

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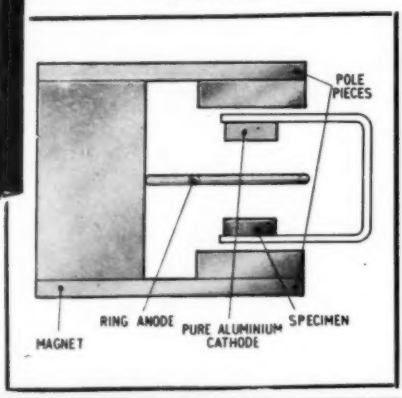

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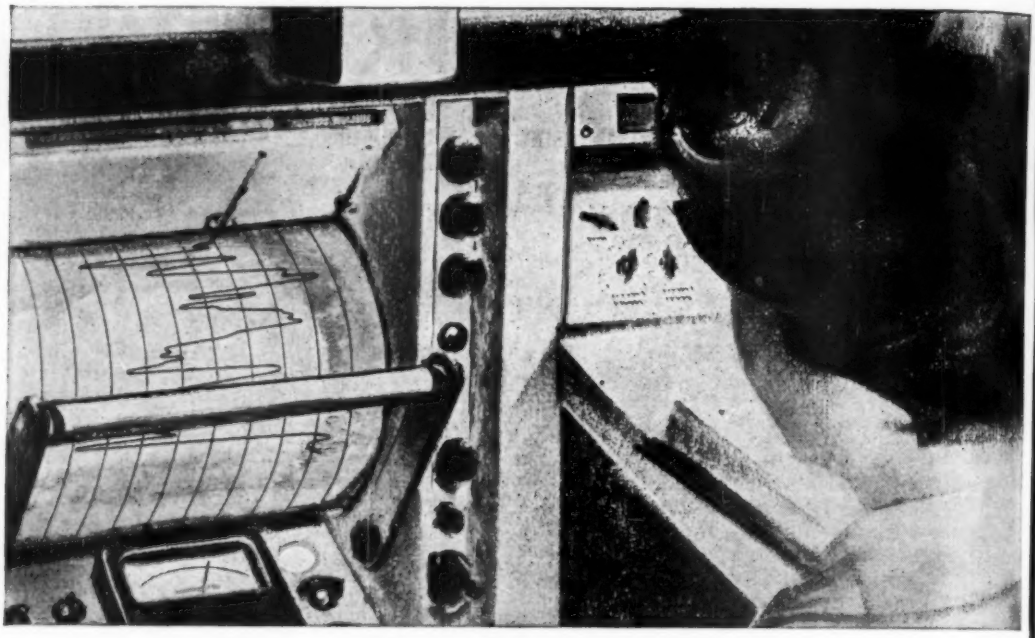
No. 4742 SATURDAY, SEPTEMBER 17, 1960 Vol. 187

CONTENTS

	Page
SUPPORT FOR UNDER-DEVELOPED COUNTRIES	971
FERRIMAGNETISM IN THEORY AND PRACTICE. By Dr. G. E. Bacon	974
ENERGY FOR THE ATMOSPHERE. By Prof. P. A. Sheppard	974
THE WORLD AROUND US. By G. R. Noakes	975
EXPLORING VECTOR SPACE. By Dr. J. W. Jeffery	975
POTASH IN WET AND DRY SOILS. By Dr. P. W. Arnold	976
PLANT TISSUE CULTURES. By A. Allsopp	976
COMBATING THE DECAY OF TIMBER. By Prof. F. W. Jane	977
ANIMAL EXPERIMENTS IN RESEARCH. By Dr. W. Lane-Petter	977
SCIENCE FOR THE FAMILY. By Dr. D. R. Dickinson	978
THE HIDDEN MIND. By Dr. Clifford Allen	978
THE IAN CLUNIES ROSS ANIMAL RESEARCH LABORATORY, PROSPECT, NEW SOUTH WALES. By Dr. Ian W. McDonald	980
RECENT SCIENTIFIC ACTIVITIES AT HALLEY BAY: INTERNATIONAL GEOPHYSICAL CO-OPERATION, 1959. By M. J. Blackwell	982
SPECIFIC INHIBITORS OF THE ADRENAL CORTEX. By Prof. J. H. U. Brown	985
OBITUARIES:	
DR. W. LAWRENCE BALLS, C.M.G., C.B.E., F.R.S. By Dr. D. W. Hill	989
PROF. J. BRONTË GATENBY. By Prof. J. N. R. Grainger	990
PROF. F. A. JENKINS. By Dr. H. G. Kuhn, F.R.S.	990
PROF. TADEUSZ MILOBEDZKI. By Dr. Wiktor Wawrzyczek	991
NEWS AND VIEWS	991
WEATHER PROBLEMS IN AGRICULTURE. By Dr. H. L. Penman	995
TUBERCULOSIS IN ANIMALS	996
THE BRITISH GELATINE AND GLUE RESEARCH ASSOCIATION	998
DOMESTIC HEATING AND THERMAL INSULATION	999
POPULATION TRENDS AMONG HAWKS AND OWLS IN ILLINOIS	999
SINGAPORE BOTANIC GARDENS	1000
ROAD ACCIDENTS DURING CHRISTMAS 1959	1000
UPPER-ATMOSPHERE WIND STUDIES BY *Skylark* ROCKET SODIUM EXPERIMENT. By Dr. G. V. Groves	1001
FATE OF TRANSFORMING DEOXYRIBONUCLEATE FOLLOWING FIXATION BY TRANSFORMABLE BACTERIA. By Dr. Maurice S. Fox and Dr. Rollin D. Hotchkiss; Dr. Maurice S. Fox	1002
EFFECT OF ACRIDINE ORANGE ON LIVING BULL SPERMATOZOA. By C. van Duijn, jun.	1006
EFFECT OF GLYCERINE ON THE X-RAY SENSITIVITY OF *Serratia marcescens*. By Dr. D. L. Dewey	1008
BIOSYNTHESIS OF CELLULOSE BY *Acetobacter acetigenum*. By Dr. A. M. Brown and Dr. J. A. Gascoigne	1010

(*Continued overleaf*)



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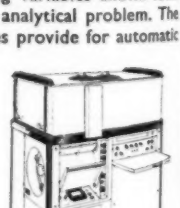

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LETTERS TO THE EDITORS

Page

PHYSICS

Electromagnetic Standing Waves and Ball Lightning.—Dr. Lewi Tonks 1013

Absorption of Ultra-violet and Visible Light by Ultra-thin Sections of Vitrinite from a High-volatile Bituminous Coal.—Sabri Ergun, J. T. McCartney and R. E. Walline 1014

Dependence on Temperature of the Interlayer Spacing in Carbons of Different Graphitic Perfection.—E. G. Steward, B. P. Cook and E. A. Kellett 1015

RADIOPHYSICS

Ionospheric Information from Satellite Signals.—Dr. G. H. Munro 1017

Observations of 'Whistlers' and 'Chorus' at the South Pole.—Dr. L. H. Martin . . . 1018

GEOLOGY

Olivine-Spinel Transition on Nickel Orthosilicate.—Dr. A. E. Ringwood 1019

Potassium–Argon Ages of Some Rocks from the South Atlantic.—John A. Miller 1019

Age of the Cambrian System.—Prof. C. F. Davidson, O.B.E. 1020

CHEMISTRY

A Molecular Complex of Benzene and Hexafluorobenzene.—Dr. C. R. Patrick and G. S. Prosser 1021

Structure of the 1 : 1-Adducts of Cupric β-Diketone Chelates with Heterocyclic Bases.—Dr. D. P. Graddon and E. C. Watton 1021

Catalytic Racemization of *tris*(Ethylenediamine)-Cobalt(III) Ion.—Dr. F. P. Dwyer and Dr. A. M. Sargeson 1022

'Eddy' Diffusion in Chromatography.—A. Klinkenberg and F. Sjenitzer; J. Calvin Giddings 1023

BIOCHEMISTRY

Exclusion of Inulin from Solutions of Hyaluronic Acid.—Dr. A. G. Ogston, F.R.S., and C. F. Phelps 1024

Alkaline C-1 Fructose-1,6-diphosphatase: Evidence for its Participation in Photosynthesis.—Dr. Robert Smillie 1024

Transiodination of Proteins during Enzymic Deiodination of Thyroxine.—Dr. J. R. Tata . 1025

Stabilization by Manganous Ions of Ribosomes from Embryonic Plant Tissue.—Dr. J. W. Lyttleton 1026

The β-D-Fucosidase Activity of Mammalian Tissues and its Relation to other Glycosidases.—Dr. G. A. Levvy 1027

Biosynthesis of Squalene by the Annelid *Lumbricus terrestris*.—Joyce M. Wootton and Lemuel D. Wright 1027

Phosphate Esters produced by Mammalian Skin from 2-Deoxy-D-glucose.—Dr. S. A. Brooks, Dr. J. C. Lawrence and Dr. C. R. Ricketts . 1028

An Antibiotic Complex derived from a *Streptomyces* and active against Gram-positive Bacteria.—B. Sgarzi, A. M. Lugli and G. Giolitti 1029

PHYSIOLOGY

Effect of Hydration on Hexobarbitone-induced Sleep in Mice.—Dr. N. K. Bhide 1030

Evidence for the Identity of Natriferin, the Frog Water-Balance Principle and Arginine Vasotocin.—Prof. Wilbur H. Sawyer 1030

Effect of Reserpine on the Nucleotide and Catecholamine Content of the Denervated Adrenal Medulla of the Rat.—Prof. Nils-Åke Hillarp . 1032

Effect of Three Compounds related to Diethylstilbœstrol on the Phagocytic Activity of the Reticulo-Endothelial System.—Prof. T. Nicol, G. Brownlee, C. Druce and C. C. Ware . . 1032

Page

Infra-red Examination of the Association of Cornea Proteins with Water.—Dr. S. Bakerman and C. Mitchell 1033

Diuretic Activity of 2-Amino-4-metachloroanilino-1,3,5,triazine (*R*-37).—D. J. Mehta, Prof. U. K. Sheth and Dr. C. V. Deliwala . 1034

A Suggested Effect on Levels of Catecholamine in Brain produced by Small Doses of Reserpine.—F. Barbara Orlans and Dr. Bernard B. Brodie; Herbert Sheppard and Joseph H. Zimmerman 1034

HÆMATOLOGY

β-Globulin Polymorphism in Chimpanzees.—Prof. Samuel H. Boyer and Dr. William J. Young . 1035

Effect of Ribonuclease on Erythrocyte Antigens.—Prof. Emanuel Hackel and Robert E. Smolker 1036

BIOLOGY

A Bleached Variant of *Euglena gracilis* showing a Doubling of the Content of Deoxyribonucleic Acid.—Dr. George Brawerman, Miss Carole A. Rebman and Prof. Erwin Chargaff . . . 1037

Plastids of the *Avena* Coleoptile.—Helen P. Sorokin and Prof. Kenneth V. Thimann . . 1038

Anastomoses in the Phycomycetes.—Dr. D. M. Griffin and Helen N. Perrin 1039

Occurrence and Function of the Provisional Embryonic Cuticle of a Lepidopteran.—Dr. R. K. Sharan 1040

Incorporation of Acetate into Protein by Obligately Parasitic and Saprophytic Fungi.—Dr. Richard C. Staples and Dr. H. P. Burchfield . 1040

MICROBIOLOGY

Adaptive Chain in the Conversion of Antimetabolites into Metabolites.—Dr. K. I. Markov, Dr. G. K. Saev and Dr. E. V. Golovinsky . 1041

A Vacuolar System in Cells of *Agrobacterium tumefaciens* as shown by Electron Microscopy of Ultra-thin Sections.—Miguel Rubio-Huertos and Paul R. Desjardins 1043

Capacity of Extracts from Prestarved *Escherichia coli* to incorporate Thymidine into Deoxyribonucleic Acid.—Dr. Daniel Billen 1044

Effect of the Peritoneal Fluid of the Guinea Pig on Strains of Enteric Bacilli.—Prof. R. J. Roantree and L. R. Collis 1045

SOIL SCIENCE

Domain or Turbostratic Structure of Clays.—L. A. G. Aylmore and Dr. J. P. Quirk . . . 1046

VIROLOGY

Polyphenol Oxidase Activity of *Nicotiana glutinosa* Leaves infected with Tobacco Mosaic Virus.—Dr. Masatake Kikuchi and Akira Yamaguchi 1048

Direct Infra-Red Spectral Analysis of the Cucumber Mosaic Virus Infection Process.—Prof. G. W. Cochran, G. W. Welkie and J. L. Chidester 1049

Site and Nature of Adenovirus Nucleic Acid.—Dr. M. A. Epstein and Dr. S. J. Holt . . 1050

ARCHÆOLOGY

The Enkomi Silver Cup.—Dr. G. F. Claringbull and A. A. Moss; Dr. Harold J. Plenderleith, C.B.E. 1051

MISCELLANY

Cost of Scientific Periodical Publications.—Capt. I. R. Maxwell 1052

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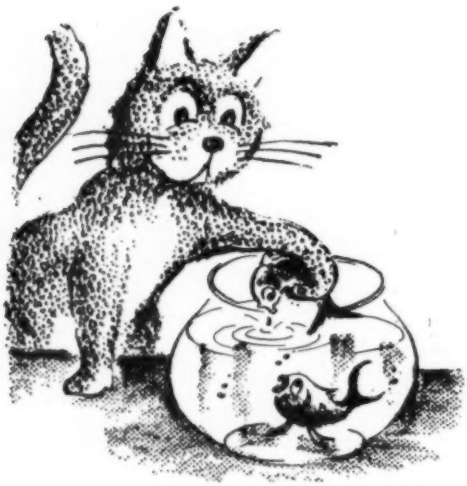

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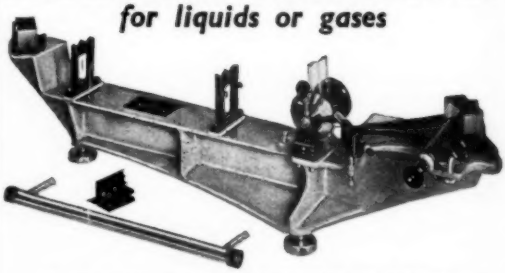

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SUPPORT FOR UNDER-DEVELOPED COUNTRIES

ONE of the most interesting, and possibly most significant, features of the annual report of the Colonial Development Corporation for 1959* is the stress laid on man-power. Some reference is made to this question in three sections of the general review of the year, although in that dealing with public relations no more is said than that a well-informed and interested staff are needed in the Corporation's work and that all staff at home and overseas are helped to realize that, while concentrating on their own particular work, they are contributing a significant part to a widespread and many-sided enterprise. The references made under management and under training schemes are of much wider significance, and illustrate the importance of much that Mr. Kenneth Younger urged in his Sydney Bull Lecture last November on "Trained Man-power for New States: the Scope for International Action", and in his subsequent study in some problems of trained manpower published under the title "The Public Service in New States"†. It might be added that Dr. E. B. J. Portman, rector of the Netherlands College for Representation Overseas, described the Dutch experiment in training staff for foreign posts to the Royal Society of Arts last January.

With enterprise so diverse in character and so widespread in location as that of the Colonial Development Corporation, it is impossible to over-stress the importance of efficient management, and in this latest annual report it is urged that such an organization can only be successful when there is full confidence in the reliability and competence of local management. The Corporation constantly gives special attention to this problem both in the selection of managers for those projects controlled directly or through subsidiary companies, and by making satisfactory expert management a necessary condition for financial participation in any development enterprise. As a result of the continued emphasis given to this aspect, the Corporation has accumulated much experience in judging the best form of management for many different kinds of undertakings, and has also built up a considerable team of trusted managers.

This management and the continued recruitment of suitable young people will only continue to be possible, however, if there is general confidence that the Colonial Development Corporation will be given the chance to grow, and if it is not constantly threatened with a contraction in its scope. Here, moreover, there are two aspects of the problem—organization and training—and both alike can affect recruitment. On the first, the report comments that important as is the selection of good management in the first instance, the process of watching the progress of that management and making changes where necessary is even more important, and it claims that the Corporation's methods of supervision through its regional organization are especially useful.

There can be no doubt from much independent criticism of technical assistance schemes that management has frequently proved one of the weakest points. This may have been due on occasion to bad selection, but more generally it is due to the lack of suitable managers or to imperfections of training. The Colonial Development Corporation is convinced that successful development of under-developed territories requires more than finance; capital investment must be guided and controlled by skilled and experienced management, and it is particularly important that development should proceed as a partnership with the local people in each country.

Through its regional organizations, subsidiary companies and projects, the Corporation is helping suitable people in British overseas territories to undergo business training to equip them to hold responsible posts in any commercial organization. Several candidates from Nigeria, Malaya and the West Indies have already received training in management in the regional and London offices of the Corporation, and scholarships at Commonwealth universities are now being offered in the West Indies and in North Borneo. Training for technical and supervisory positions is being given on projects in the West Indies, the Far East and in various parts of Africa.

In this field, therefore, the Corporation is making a contribution to educational co-operation in the Commonwealth that is by no means negligible, and which underlines both the urgency and the importance of the task of education in the dependent and newly independent territories alike. Mr. Younger's lecture, and even more his book, help to put it in a wider perspective. Both the lecture and the book are concerned primarily with the new States, and accordingly with a field where the Colonial Development Corporation, for example, does not operate. Apart, however, from the fact that these States, most of which are former Colonial dependencies, face many of the same problems as all under-developed territories, the newly independent States have usually a fairly extensive trained administration, consisting largely of expatriates, and their national life and economy are geared to the existence of this corps of trained administrators, many if not most of whom are likely to leave, at or soon after, independence.

These trained administrators cannot be replaced immediately, and Mr. Younger is not concerned simply with the problem of educating and training the people of these States to fulfil such positions, or

* Colonial Development Corporation. Annual Report and Statement of Accounts for year to 31.12.59. Pp. v+77. (London: H.M. Stationery Office, 1960.) 5s. net.

† The Public Service in New States: a Study in Some Trained Manpower Problems. By Kenneth Younger. Pp. viii+113. (London: Oxford University Press, 1960.) 12s. 6d. net.

with the special problem of maintaining relatively modern and sophisticated services during a vital transitional period before such indigenous trained man-power is adequate. He is equally concerned, more especially in his book, with what could be done before independence is reached to ease this transitional period. Moreover, while the major burden of creating a supply of trained man-power must inevitably fall on the new States themselves, which cannot escape the long process of building up a population generally educated for the needs of a modern society, Mr. Younger points out that the process can be accelerated by the conscious adoption of certain priorities in education and training, and that substantial assistance can be given from outside.

This, Mr. Younger believes, should be one of Britain's main pre-occupations with her overseas territories, and it is of course one of the main purposes both of the Commonwealth Scholarship and Fellowship Plan and of the other measures of Commonwealth educational co-operation resulting from the Commonwealth Education Conference. Like Dr. B. V. Bowden, of Manchester, he points out that even where there is a substantial element of trained man-power, as in Ghana, it is ill-balanced ; and while there may be a full or excessive complement of lawyers alongside a desperate shortage of scientists and engineers, even the existing supply of the latter may be ineffectively used for lack of sufficient technicians or craftsmen. Little has been done until recently to correct the tendency of young scholars in such territories to prefer the arts to science ; but the measures resulting from the Commonwealth Education Conference offer a much better opportunity of correcting their preference by planning higher studies and overseas scholarship programmes with the view of meeting the most pressing needs of particular territories.

Discussing more particularly this transitional period, Mr. Younger points out that while the extent to which expatriate long-service administrative officers and professional and technical officials have been replaced by locally recruited men in the independent territories varies from one territory to another, it rarely much exceeds 50 per cent and is frequently much less in the senior grades. The ideal solution in such circumstances is a phased run-down of the old service, as locally recruited men become available, and this may take anything from three to fifteen years. The process has been successfully worked in the Federation of Malaya and was not unsuccessful in Ghana, while the prospects for Nigeria are reasonable. Success is only possible in favourable political circumstances. In Indonesia, the Sudan and Guinea, for example, a sound relation between the new Government and the old Colonial Service was unattainable for political reasons, and sources of man-power had to be sought apart from the former Colonial power.

No international organization yet exists on a scale which would enable it to step in and make good major deficiencies in trained man-power in an emergency, and Mr. Younger in his lecture suggests that such situations could be far more advantageously handled if international organizations were suitably expanded and strengthened. Once this transition is past, new States are on the same footing as all other underdeveloped territories and have to build up education at all levels, increasingly in their own countries, and with their own teachers. Mr. Younger stresses this indigenous aspect strongly. While he emphasizes that Britain deserves much credit for creating the existing institutions of higher education in Africa, he points out that these are only a small nucleus of what is required, and he has formed the impression that the Governments of highly developed nations, for example, the United States, Britain and France, find it easier to provide scholarships in their own institutions than to give help in developing local institutions.

Whether this is true or not—and it could in part be a consequence of the shortage of trained man-power, especially of scientists and technologists in these countries—the United Nations Technical Assistance Administration has undoubtedly found, in the promotion of training facilities on the spot in various areas, a field which has been neglected by others. Considering the relatively small scale of United Nations activity which Member States have so far been willing to finance, Mr. Younger thinks the United Nations can claim to be making an outstanding contribution in this limited field, although its current annual budget for the Expanded Programme of Technical Assistance is only just over 33 million dollars, supported, however, by substantial counterpart funds in local currency from recipient Governments. Further, successful projects tend to set in motion activities eventually out of all proportion to the initial cost. Some 86 countries have contributed to the programme, and 140 territories have benefited from it : in 1958 experts were drawn from 64 different countries and senior men were sent for training to 68. The experts are mostly international officials, paid by and answerable to the United Nations, and the service has been fairly stable in size for several years. It differs from the more recent Operational and Executive Service of the United Nations, now known officially as Opex, put into operation in 1959 with a maximum of 25 officials, who go out as employees of the Government requesting assistance.

Mr. Younger does not foresee a spectacular growth of this service on the purely administrative side, but on the professional and technical side it might grow considerably and the demand remain steady almost indefinitely. He suggests, therefore, that it should be given some relative permanence, so that it could hope to include a proportion of men who make international overseas service their career and consequently become genuinely expert in its techniques. It would, he suggests, be little if at all easier for Britain than for the United Nations to provide a constant stream of first-class professional and technical men on contract, which is the main requirement to-day. In West Africa, for example, an international body

which could build up a corps of experts familiar with the problems of the whole area and capable of transcending the artificial boundaries dividing the former British and French Colonies would have a real advantage. To treat West African medical, agricultural and forestry problems as a single whole would economize in man-power and serve as a useful antidote to 'Balkanization', which is one danger threatening the area after the end of Colonial rule.

It is in this context and that of the proved success of the United Nations in setting up institutes and schools of public administration in South and Central America and in helping to create similar establishments in Egypt, Ethiopia, the Sudan and Turkey, and providing skilled help and advice in this field in other countries of Asia and Africa, that Mr. Younger raises the question whether the Member States of the organization are now willing to turn technical assistance into a major force in world development or not. He thinks that new States would welcome an increase in multilateral aid, but they cannot be expected to press for it so long as it is evident that those Powers, which have both capital and skilled man-power to contribute, are unwilling to supply through international channels more than a fraction of the assistance they offer on a national basis. Nevertheless, he suggests that in the matter of trained man-power, the long-sighted policy would be deliberately to build up international services, both world-wide and regional, as the instruments best suited to future needs, and to the conditions of the post-Colonial period.

Mr. Younger's book describes a study undertaken at the request of Nuffield College, Oxford, and the Leverhulme Trust and based on a visit to Ghana and Nigeria in June and July 1959. While it is largely concerned with the public services in Nigeria and the Southern Cameroons, its clear analysis, including a detailed discussion of the movements which took place in Nigeria and Ghana, is designed to ease the transition for territories reaching independence in future, and to suggest what more might be done beforehand to that end. The difficulties and obligations of the Colonial Power are fairly discussed, but to the professional man the great interest of the book lies in its discussion of the future role of the Overseas Service, and the possible forms which such a Service could take if it is to encourage Civil servants, including scientists and other professional men, to remain in the overseas field. Even for professional and technical officers whose services are likely to be needed longer than those of their administrative colleagues, he is doubtful whether such a Service can be provided on a Commonwealth basis. Adequate career prospects, he suggests, can now be found only in an international organization.

While Mr. Younger considers that it would be sound policy for Britain to participate increasingly in the work of the United Nations and other international bodies in this field, this is not the only possibility. The launching of the British Overseas Civil Service has largely been frustrated through the anxiety as to whether the United Kingdom Government now has direct control over a sufficient range of territories to be able to guarantee a life-time of employment abroad. If, however, ways were found of encouraging men, whose careers would basically be pursued at home, to regard periods of overseas service as a normal and advantageous feature of their professional lives, this difficulty would disappear. Furthermore, it is easier for a technical man to keep abreast of modern knowledge if he retains his home base than if he spends his whole career in territories on the periphery of technical advance.

Present arrangements do no more than make it just possible for a keen man in the British public service to serve abroad for a period, albeit at some risk to his career, though a start has been made in the field of education to improve this. If, however, encouragement of overseas service were explicitly adopted as a part of government policy, a big change could probably be effected at very small cost. As Mr. Younger points out, it is primarily a change in attitude that is required, and especially in that of the Civil Service in Britain. Apart from this, he suggests the creation of an agency in Britain outside the framework of Colonial administration, which can give help to dependent and independent areas alike, and by pursuing simultaneously both these methods Britain would be making the best use of her accumulated experience and contributing at the same time to international co-operation and to the stability and development of new States.

Very clearly some fundamental thinking, as well as a change of attitude, is required, if Mr. Younger's suggestions are to be adopted and errors of the past corrected. It is not simply a matter of education policy and development in the Commonwealth, or even of the more effective training of staff proceeding overseas, either in the Oversea Civil Service or in private employment. The contribution and function of the Colonial Development Corporation must obviously be taken into account, as well as that of Barclays Bank D.C.O., Unilever, Ltd., and other firms that take great care in training staff for overseas; but besides the Government responsibility for seeing that the conditions offered for service overseas are reasonable and attract officers of the highest quality, it is also important that the quantitative demand, especially for scientific and technical officers, should receive due attention in assessing the demand for scientific and technical man-power and in determining the provision to be made to meet that demand. This was a notable omission in the last report on scientific and engineering man-power in Britain, and the omission indicates one of the failings of Government policy to which Mr. Younger directs attention. His criticism, however, is on the whole so constructive that it is to be hoped that the Government will respond swiftly to the challenge it offers, and provide alike the increased resources and the measures which the new situation demands, the critical importance of which has been underlined by all the subsequent events in the Congo.

FERRIMAGNETISM IN THEORY AND PRACTICE

Ferrites

Physical Properties of Ferromagnetic Oxides in Relation to Their Technical Applications. By J. Smit and H. P. J. Wijn. Pp. xiv+369. (Eindhoven: Philips' Technical Library, 1959.) 72*s*.

THE history of the growth of our knowledge of ferrites offers an excellent example of the conditions which are becoming increasingly necessary for success in present-day physical research. Following Néel's original theoretical conceptions of ferrimagnetism, the study of these materials was carried on mainly in an industrial organization, furnished not only with the necessary technical prowess to ensure proper sample preparation and purity control, but also endowed with a liberal policy towards basic research. Alongside this work the key to a fuller understanding emerged from government-sponsored laboratories, by means of the technique of neutron-diffraction which had grown up as a by-product of the operation of nuclear reactors. As a result of this combined attack by physical, chemical and crystallographic methods, many of the properties of ferrites are now much better understood than those of the ferromagnetic metals.

Against this background Drs. Smit and Wijn give an account of the basic magnetic properties of ferrites, emphasizing the properties which are of importance for practical applications and with particular reference to the chemical composition and crystal structure of their materials. They emphasize that they are writing at an intermediate level for readers who are actively working with ferrites, and especially in connexion with their technical uses. As a result the opening chapters, which as Part *A*, "Theory", form almost one-third of the book, concentrate on explanations given in terms of simple physical models rather than strict mathematical demonstrations. Many readers will indeed welcome this and will find that these early chapters offer a very readable account of current conceptions in magnetism, viewed in a field much wider than that of ferrites and presented in an original manner.

Succeeding sections of the book are Part *B*, "Methods of Measuring the Ferromagnetic Properties", and *C*, "Intrinsic Properties", which considers in turn the three classes of ferrite with spinel, hexagonal and garnet structures respectively, and describes their chemical composition, crystal structure, saturation magnetization and crystalline anisotropy. One can complain that the garnet class—particularly those containing rare-earth oxides—get rather scant mention compared with the hexagonal group, where the authors do justice to Braun's elegant work which unravelled the structural secrets. In the last third of the book, Part *D*, entitled "Polycrystalline Ferrites", we come to the more purely technical data for the sintered polycrystalline product as normally handled, a macroscopic product the mechanical and electrical properties of which will depend on porosity and any crystallite orientation induced during the manufacturing process. An idea of the very comprehensive discussion in this section is provided by some of the section headings. There are, for example, sections on the frequency-dependence of the conductivity, dielectric constant, permeability and magnetization curve and on the effect of mechanical stress on the magnetic spectrum and the hysteresis loop. The data here, as indeed throughout the book, are presented very clearly in graphical and tabular form.

The book is excellently produced and pleasant to read, though perhaps with more than its fair share of trivial proofing errors which have escaped detection. As a personal feeling I must admit to a dislike of the system of referencing, using forms such as *Ab* 1 and *Ze* 1 which necessitate turning to the back of the book in order to discover the year of any particular publication. Finally—as a suggestion—the less-specialized reader, who may peruse a good deal of this book with profit, might welcome a few notes on the actual employment of ferrites in electronic devices.

G. E. Bacon

ENERGY FOR THE ATMOSPHERE

Turbulent Transfer in the Lower Atmosphere

By Dr. C. H. B. Priestley. Pp. vii+130. (Chicago: University of Chicago Press; London: Cambridge University Press, 1959.) 28*s*. net.

THE meteorologist is vitally concerned with the rate at which energy enters or leaves the atmosphere at the surface of the Earth because this flux ultimately provides the major control on the behaviour of the atmosphere up to quite high levels. Momentum is also drained from the atmosphere at the surface; while man, beast and plant lives (or fails to live) in the layer of air immediately affected by these fluxes. They are mainly, for momentum and latent heat wholly, effected by atmospheric turbulence, except at the very boundary, and Dr. Priestley has written a monograph describing the present position of the study of these turbulent transfers. As the leader of a notable group on the subject near Melbourne over most of the post-war period he is admirably fitted to undertake the task.

Priestley begins at the stage reached by the subject in the early post-war years, as expounded by Sutton in his "Micrometeorology". He sets out to provide a coherent body of knowledge on the relation of the vertical fluxes of heat, matter (evaporation) and momentum to the 'external' parameters—the choice of these is quite a part of the problem, particularly as they are affected by the density or thermal gradient, or, from the other end of the telescope, by the heat transfer itself. The simplest condition, that obtaining when the transfer properties of turbulence are not significantly affected by buoyancy forces (non-adiabatic lapse-rate), is now moderately well understood and described, though the flux of heat or water vapour has not yet been adequately related to the actual surface temperature or vapour pressure. For heat, a second special case obtains when the transfer properties of turbulence are dominated by buoyancy forces to the exclusion of shear-engendered forces—the regime of free convection—and here Priestley's dimensional analysis of the problem and the observations of his group provide the required relations. In the general case, however, where both the above forces are effective and all the fluxes are concerned, theory is somewhat halting, but a framework for observation has been provided and the Melbourne observations of fluxes begin to fill the frame. If Priestley is correct, this general case is not of outstanding practical importance for

upward heat transfer because he argues for a quite narrow zone of conditions between the regimes on either side.

The evidence on these matters is skilfully assembled and discussed by the author. He also presents a theory of buoyant motion which is cogent and well supported by the experimental record so that mechanism begins to appear. The bridge has yet, however, to be built to the more fundamental approach adopted in recent years by W. V. R. Malkus in which disposable constants do not appear—this work is not referred to by Priestley. Not the least valuable part of the author's work is to relate the surface fluxes to the larger-scale motions of the atmosphere and to the problem of air-mass modification.

This is an excellent monograph for those with some familiarity with the subject of turbulent transfer, not necessarily in the atmosphere. It is thus to be recommended not only to meteorologists but also to practically all fluid dynamicists concerned with boundary layer problems. They will find writing which is primarily physical in approach and only so much mathematics as is needed to make the physics effective. The writer is a notably good apologist for his own cause, and the reader's enjoyment and stimulus should come in part from reacting to the writer's essence. No serious misprints have been noted and the book is nicely produced.

P. A. SHEPPARD

THE WORLD AROUND US

The World around Us

Edited by Sir Graham Sutton. (Six Essays based upon the Christmas Lectures delivered at the Royal Institution, 1958.) Pp. vi+122. (London: The English Universities Press, Ltd., 1960.) 16*s*. net.

THE 1958–59 Christmas Lectures at the Royal Institution were planned to mark the close of the International Geophysical Year, and this collection of essays based on the Lectures tells of the kind of work that geophysicists do, and describes some of the projects undertaken during the Year. I have never been able to understand the difference between a 'juvenile auditory' and 'teen-agers', or why it is that the former flock eagerly to hear lectures of the highest excellence by outstanding scientists, and the latter are cynically fobbed off with commercialized intellectual fare which could be described as abysmal if it were not certain that there are lower depths yet for future dredging. The answer is, I suppose, that chance is a fine thing. The regular publication of the Lectures brings them annually before a wider and equally eager audience; and as a most attractive book at about half the price of a long-playing record, this present volume should become the best-seller it deserves to be. It gives young people what they really want when they get the chance to choose it.

The first essay, by J. A. Ratcliffe, describes the exploration of the electron density in the ionosphere by radar echo-sounding, satellite transmissions, and observations of whistling atmospherics. J. M. Stagg describes the general features of the Earth's magnetic field, the complex interplay of surges in the Earth's liquid core and effects from outside, particularly the emissions from solar flares. R. L. F. Boyd writes on the information obtained about the upper atmosphere from rocket experiments and instruments carried in rockets and satellites. Sir Graham Sutton, who has edited the book, deals himself with the lower atmosphere and its weather. G. E. R. Deacon's account of oceanography presents a general picture of the scope of a science that ranges from the detailed recording of variations in mean sea-level to harbour design, tidal waves, and the tracking of deep sea currents. G. de Q. Robin describes the problems of establishing and maintaining an antarctic base, and the work done at Scott Base as part of the programme of the International Geophysical Year.

The authors and editor are to be congratulated on transmuting the Lectures into essays that reach the highest level of popular interpretation. They contain so much information that is really new to the ordinary scientist who has not followed the march of events closely, and should be widely appreciated on this account as well. The publishers also must be congratulated on the production. G. R. NOAKES

EXPLORING VECTOR SPACE

Vector Space and Its Application in Crystal-Structure Investigation

By Prof. Martin J. Buerger. Pp. xiv+347. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 96*s*. net.

PROF. D. KOSAMBI, the Bernal of India, has written a collection of articles entitled "Exasperating Essays". It would make a very suitable second title for Prof. Buerger's "Vector Space and Its Application in Crystal-Structure Investigation". The book is exasperating because the subject is of vital concern to X-ray crystallographers, endeavouring to piece together the jig-saw puzzle of their experimental data, and yet one cannot help feeling that the really significant aspects could be presented in a smaller compass and made easier to assimilate.

The crystallographers' jig-saw puzzle, when the pieces are finally fitted together correctly, gives a picture of the electron-density distribution in the crystal, the peaks corresponding to atomic positions. Unfortunately the pieces will fit together in many different ways, and the key, the phase angle of the diffracted X-ray beam or the number on the back of the piece, which would enable the picture to be built up directly and systematically, has been obliterated. There is no means yet known by which the relative phases of diffracted X-ray beams can be determined directly, and it is this 'phase problem' of the structural crystallographer which is responsible for the study of 'vector space'. The concept originated in 1934 when Patterson showed that a modified jig-saw puzzle could be constructed directly from the experimental data, but that the picture so constructed was the 'vector set' derived from the actual atomic arrangement. That is, all the possible vectors between atoms are constructed and one end of each is then transferred to the origin. In general, there are n^2 peaks in the vector diagram derived from n atomic positions, and the problem resolves itself into the 'unsquaring' of this distribution of vector peaks.

Prof. Buerger has played a prominent part in the gradual development of systematic methods of tackling this problem, and the present book presents a survey of these methods, many of which the author has himself originated. It may be said that the book is too complete and insufficiently critical, but another

of its exasperating aspects is that one cannot really apportion any blame for this. It is by no means yet clear which methods are going to be most fruitful in exploring vector space, and very little beyond an intuitive idea that the two methods are more or less equivalent has yet been said on the relation of vector methods to the alternative determination of phases by the statistical analysis of the raw data. In these circumstances, to have all the fundamental ideas on vector space and the various analytical methods brought together (and developed) in one book is really as much as one has a right to expect, and something for which all X-ray crystallographers owe Prof. Buerger a debt of gratitude. The criticisms that one has are largely silenced and, exasperating or not, this book will be required reading for all those concerned with Patterson analysis for a long time to come.

J. W. Jeffery

POTASH IN WET AND DRY SOILS

Japanese Potassium Symposium

Nine papers read at the First Japanese Congress, Tokyo, 1957. Pp. 137. (Berne: International Potash Institute, 1959.) n.p.

Potassium Symposium, 1958

Twelve papers read at the Fifth Congress of the International Potash Institute (4 Sessions), Madrid, 1958. Pp. 426. (Berne: International Potash Institute, 1959.) n.p.

SINCE its inception in 1952, the International Potash Institute (Berne) has rendered a valuable service to agriculture and agricultural research. As well as issuing the monthly *Potash Review* leaflets in four languages, the Institute has organized five potassium congresses in different European capitals over the years 1954–58. The Institute also instigated a Japanese potassium symposium, held in 1957; the nine papers presented in Tokyo are now reprinted in English. Four of the papers deal, not surprisingly, with potash and the rice crop. Students of rice culture will find factual articles which range from fertilizer usage to the relationships between potassium nutrition and the disorders caused by fungus and bacterium attacks on the crop. A paper on fruit trees deals specifically with the effects of fruiting on the composition of young orange tree leaves. The nutrition of tobacco is discussed in terms of deficiencies and excesses of potassium on the intake of other nutrients. Recent work on the distribution and lability of potassium in cells and the effect of different potassium-levels on respiration is also described. The reasons for the variations in the potassium-supplying power of volcanic ash soils are examined; usually the parent volcanic ash contains rather little total potassium, which is lost rapidly on weathering. The main products of weathering, allophane, halloysite and gibbsite, do little or nothing to help the potassium economy of such soils. All the papers seem to be well documented.

The volume entitled "Potassium Symposium, 1958", contains the proceedings of the fifth congress of the International Potash Institute, held in Madrid. Following an introductory paper on Spanish agriculture and the use of fertilizers, most of the contributions impinge on the general theme of "Potash and Water". Special attention is given to the potash manuring of Spanish irrigated areas and potassium problems related to the mineralogy of Spanish soils. Three articles deal with potassium, the plant and the water regime; these include treatments of the morphological and physiological conditions associated with drought resistance, the influence of potash on the water economy of plants and the effects of extracts of rotted straw on the potassium uptake and physiology of plants. Other articles deal with potassium uptake and the moisture status of soils (England), potassium in saline soils (Spain) and the importance of potassium in saline irrigation water (Israel). The papers presented towards the end of the Congress allow comparisons of the problems of potash fertilization in temperate, arid and wet-tropical climates. The development of fertilizer usage in the Hawaiian sugar industry is also described; here potash-urea pellets are now hailed on the cane from the air. Readers will find short articles on the use of potash in Portugal, chlorosis and calcium/potassium ratios and the use of potash on rubber trees.

The main articles, which are well documented, are all summarized in four languages.

Having circumscribed the globe, one's thoughts gravitate towards the numerically enormous number of the world's farmers who have yet to open their first bag of potash. The International Potash Institute will, year by year, make these fewer and so help mankind towards a happier existence by encouraging the proverbial two blades to grow where one grew before.

P. W. Arnold

PLANT TISSUE CULTURES

La Culture des Tissus Végétaux

Techniques et Réalisations. Par Prof. R. J. Gautheret. (Ouvrage publié avec le Concours du Centre National de la Recherche Scientifique.) Pp. iv+884. (Paris: Masson et Cie., 1959.) Cartonné toile, 10,500 francs.

FOR a considerable time following the first successful culture of plant tissues in 1934, further studies of such materials were carried out mainly by a few pioneer workers. During recent years, however, there has been a great widening of interest in plant tissue cultures, which are now no longer treated merely as curiosities but are utilized in the investigation of a wide range of botanical and related problems. The scattered information obtained from these recent and earlier studies has now been collected in one comprehensive volume, and it is fitting that this task should have been carried out by Prof. Gautheret, who was not only the first worker to achieve the culture of plant tissues but by his own work and that of his pupils has also continued to make notable contributions in this field.

As the title indicates, the book deals only with tissue cultures, and the allied subject of organ culture is mentioned only incidentally. The first section, of more than 200 pages, covers the important topic of technique. The media and methods employed by various workers are described in full detail, perhaps even unnecessarily so; the reader is given several plans of the arrangement of instruments on the work table. However, such detailed instructions have the advantage of giving even the beginner in this line of work a good chance of success.

The second and largest part of the book is concerned with morphogenesis in plant tissue cultures. A detailed description is given of the behaviour of

tissues when first placed in culture, and of the morphology and development of established strains. There is also an excellent treatment of the differentiation of tissues and organs in various cultures, an aspect of plant tissue culture which has probably yielded the most interesting results to date.

Other sections deal with the utilization of tissue cultures in various special studies. Thus, the use made of tissue cultures in the study of polarity and induction is described. This is followed by an extensive consideration of the nutrient requirements of plant tissues. It is pointed out that although the tissues of many plants can be grown on purely synthetic media, other tissues can only grow, or grow better, following the supply of various plant extracts such as coconut milk. This section includes a full account of the attempts at isolation of the active factor or factors, accompanied by a careful analysis of the difficulties encountered in such work. In this connexion, Prof. Gautheret emphasizes the necessity of working with a suitable tissue strain, of known behaviour, as assay material. In this type of work, as in various metabolic studies of tissue cultures, it is important to note that freshly excised tissues show marked metabolic disturbances and may differ considerably in behaviour from established stocks.

Other topics dealt with are the culture of isolated cells, which is a very recent development, and the role of tissue cultures in plant pathology. With reference to the latter section, mention may be made of the use of tissue cultures in the culture of obligate parasites, and of a particularly interesting account of plant tumours.

In sum, this book is a fine comprehensive account of all aspects of plant tissue cultures. For a work of its size it is remarkably up to date, a supplementary bibliography including most of the important literature issued during 1958. It is well illustrated, the 438 figures including photographs, diagrams and line drawings. Although bulky, the book is easy to use, a clear subdivision of the contents facilitating the location of any particular topic. There is also a useful table of the principal established strains of plant-tissue cultures, which includes a summary of any special nutrient requirements of the various tissues.

A. Allsopp

COMBATING THE DECAY OF TIMBER

Decay of Timber and its Prevention

By K. St. G. Cartwright and W. P. K. Findlay. Second edition. Pp. xv+332+57 plates. (London: H.M. Stationery Office, 1958. Published for D.S.I.R.) 27*s.* 6*d.* net.

DECAY of timber must have been a perpetual problem since man first began to use wood, and a serious one as soon as wooden structures became elaborate. We know of the trouble and expense which faced the Navy as a result of the ravages of dry rot in the days of wooden ships, while in buildings decay of timber began to receive serious attention at the beginning of the nineteenth century, coincidently, no doubt, with the decline in the use of the traditional oak in favour of less-durable coniferous woods.

Nevertheless, with the exception of Marshall Ward's "Timber and some of its Diseases", which was based largely on German work, "Decay of Timber and its Prevention", by K. St. G. Cartwright and W. P. K. Findlay, of which the first edition appeared in 1946, seems to be the first comprehensive account of the subject to be published in Britain. It embodies much material based on the authors' investigations at the Forest Products Laboratory at Princes Risborough.

Following introductory chapters on decay in wood, and on the causal organisms, there are detailed accounts of the principal rots of standing trees, accompanied by useful tables concerned with macroscopic and microscopic features of the rots and the characteristic features, in culture, of the causal fungi. Felled timber and wood in outdoor use receive similar treatment, as does constructional timber in buildings and elsewhere. Preventive measures are described, particularly in reference to felled and converted timber and to timber in use. Decay in plywood, wood pulp and building boards receives consideration, and there is a valuable summary of present knowledge of stains and discoloration of timber. There are numerous illustrations, and those of the characteristic appearance of certain rots make a specially valuable supplement to the text and should greatly facilitate identification.

Although a comprehensive treatise, there are times when the information sought is scant and vague. This is no reflexion on the diligence and care of the authors, but rather on the large gaps in knowledge of these interesting and troublesome organisms and their activities. It is indicative of the enormous field of research which awaits the investigator, more particularly with reference to the rots and staining fungi of tropical areas. For any such research the book in question is certain to be a standard work of reference.

F. W. Jane

ANIMAL EXPERIMENTS IN RESEARCH

Achievement

Some Contributions of Animal Experiment to the Conquest of Disease. By Dr. Geoffrey Lapage. Pp. viii+255. (Cambridge: W. Heffer and Sons, Ltd., 1960.) 18*s.* net.

THIS is an unexpected, original and wholly admirable book. It could have been attempted by a journalist with a smattering of science, but it was, in fact, written by a scientist who has a considerable ability for writing. In view of the seriousness of the subject this is a piece of good fortune for the reader, while implying no derogation of the art of journalism.

Animal experimentation is not often discussed in respectable company; not often enough, and still less often without generating emotion. Dr. Lapage's book is in the direct line of descent from earlier books on the same subject by Stephen Paget and Leonard Rogers; but the treatment is entirely different. Nowhere is the word 'vivisection' mentioned, and nowhere does the author indulge in polemics or argue the moral case. He is content, and so may the reader be, to tell how animal experimentation has led, as no other method had previously led or could have led, directly to advances in human and animal medicine and their related fields of research. Among

the animals used, man himself, meaning nearly always the investigator, has a prominent place in this book, which is as worthy a way of paying tribute to our great men as we could wish for. The author has drawn freely on articles appearing in *Conquest*, the journal of the Research Defence Society, over the past ten years.

A former university lecturer who has written a standard work on parasitology might be expected to have written in the style of a text-book; but Dr. Lapage has avoided this pitfall, without sacrificing accuracy and extensive documentation. The book is outstandingly readable; yet it includes nine pages of bibliography and a further nine of index.

There is so much to applaud in this dispassionate treatment of an emotive subject that even to suggest imperfections might be thought ungracious. The emphasis on infectious diseases—three chapters out of ten—is easily defended, for they represent medicine's greatest achievements to-day. Chemotherapy, hormones, nutrition and deficiency diseases are, *inter alia*, also well treated. The absence of a foreword is regretted, all the more because the preface is so short. I discovered only two misprints.

The wise refusal of the author to discuss the moral question is the best reason of all why this book should be compulsory reading for all who recognize that, in animal experimentation, a moral question exists.

The presentation conforms to the high standard one has come to expect from the publishers.

W. Lane-Petter

SCIENCE FOR THE FAMILY

Science

Chemistry, Physics, Astronomy. Editorial Board: Dr. J. Bronowski, with Sir Gerald Barry, James Fisher, and Sir Julian Huxley. (The Macdonald Illustrated Library.) Pp. 367. (London: Macdonald and Co. (Publishers), Ltd., 1960.) 45*s*. net.

THIS is the first volume of a library of knowledge written for young readers and their parents. The book is so arranged that each double page forms a separate article. This arrangement has distinct advantages over an alphabetical one, and considerable care and thought have been taken in planning the order of the articles so that the book presents a coherent whole. There are a comprehensive glossary as well as an index. In addition, several useful references occur incidentally in the list of illustration credits; but, in a book where much of the information is of necessity superficial, a more systematic bibliography would surely have been welcomed by many readers.

The text is copiously illustrated with excellent diagrams, prints and photographs, containing information complementary to that of the text. There are also several full-page illustrations in colour by Hans Erni which, while being delightful and of high artistic merit, seem unlikely to aid the young reader in his understanding of the subject-matter.

The book unfolds the story of the advancement of the physical sciences from very early times up to the present day, including topics such as polymers, atomic power, television and satellites. A judicious and comprehensive selection has been made, and wisely no attempt has been made to introduce subjects which could not be explained adequately in elementary terms. There is a commendable lightness of touch (not always to be found in works of an encyclopædic nature) in the text, though occasional lapses do occur. For example, we are told on p. 126 that "work is done only when the force acting on a body moves that body in the direction of the force". On p. 128 the reader is apparently encouraged to use "weight" as synonymous with "mass". It is stated on p. 232 that, for a convex lens, "nearer the center ... the rays are bent less sharply" and on p. 233, for the same lens, that "the lens bends rays that pass near its center more than rays that pass near its edge". The machine shown on p. 121 has mechanical advantage 3, and not 4 as stated. Very few misprints were detected, but the young British reader needs warning that, the book being "international in scope", an American system of spelling has, perhaps unfortunately, been adopted.

These are only minor criticisms. The distinguished editorial board, and all others concerned in its production, are to be complimented on a volume of high quality which will undoubtedly give hours of enjoyment to many readers both young and old. It would surely prove a popular addition to any school library.

D. R. Dickinson

THE HIDDEN MIND

The Wish to Fall Ill

A Study of Psychoanalysis and Medicine. By Karin Stephen. Pp. vi+238. (Cambridge: At the University Press, 1960.) 10*s*. 6*d*. net; 1.45 dollars, paper bound.

THIS book is an excellent introduction to psychoanalytical theory. It has been based on lectures given to medical students at the University of Cambridge and so is comprehensible to those who have little or no psychiatric knowledge. The author draws on her clinical experience and the cases she quotes add interest to the theoretical descriptions.

Dr. Stephen commences with a discussion of the origin of the unconscious in disappointment, conflict and repression, and describes how this is solved by illness. She shows the obstacles in the way of investigating the unconscious and how psychoanalytical technique uses free association to penetrate this barrier.

Having shown the difficulties to investigation she passes on to describe what is found once we are able to lift the lid of the mind, as it were, and see what is underneath: the primitive sexual nature of the unconscious, infantile pleasure-seeking by mouth, excretory pleasure-seeking and phallic-pleasure seeking.

This naturally leads on to the fears of being deprived of such enjoyment—to the Oedipus complex and castration fears; the arousal of anxiety and guilt, and defence mechanisms; finally, the use of transference in treatment.

Many people, even now, have little or no idea what the analyst is trying to do or how he produces cures. Properly conducted analysis is much more scientific than is often appreciated and the process is more related to observing any other natural phenomena than to patting someone's hand and telling him all is well—which is the popular idea of the process.

Clifford Allen

Nature and Man

By John Hillaby. (Progress of Science Series, No. 9.) Pp. 64+28 plates. (London: Phoenix House, Ltd.; New York: Roy Publishers, 1960.) 9*s*. 6*d*. net.

ANY series of books designed to help young people to keep up with the progress of science deserves encouragement. In one of this new series, John Hillaby, European science correspondent of *The New York Times* and zoological correspondent of *The Guardian*, has attempted to show the perpetual struggle between man, animals and plants and how the balance of Nature often becomes greatly disturbed from what seem trivial beginnings. From his travels in many parts of the world Hillaby has seen the awful results which sometimes follow the upsets to natural life and records these in tense and dramatic style.

Among the topics described are the blocking of the Congo River by the devil's lilac and the expenditure of £3 million on chemicals to clear it for traffic; the fearsome effect of too many lemmings, musk-rats and rabbits and other animals; soil-erosion (unfortunate, perhaps, that Hillaby had to go to Athens to witness a soil-storm when his own native Pennines are being deposited in Sheffield and other cities); and the consequences of over-shooting big game.

There is also a chapter on the ways in which young people can train for careers in the conservation of Nature. Whether the author is right to pitch a book with this approach to young people of "12 and upwards" is very questionable. In many secondary modern schools the suggestion that Elton's "Animal Ecology" could form useful further reading for children of this age would receive a not unjustified rejection.

T. H. HAWKINS

Principles of Sugar Technology

Edited by Pieter Honig. Vol. 2: Crystallization. Pp. xviii+567. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1959.) 95*s*.

THIS volume was produced by a selected group of experts as with the first volume. There is a vast mass of useful material to be found in these pages and there is little to be criticized. The crystallography of sucrose might have been improved by the incorporation of some of the useful work done in the University of Bristol, namely, by N. Albon and W. J. Dunning (see *Nature*, **180**, 1348; 1957). This was on growth steps of sucrose crystals. Further work was not then published, but is to be found in the thesis of N. Albon at Bristol. Also in Doremus, Roberts and Turnbull (Wiley, 1958) and in *Acta Crystallographica*, Honig directs attention to the loss by inversion on solutions above 65° Brix, as being greater than given by the Stadler tables. He also mentions that pan work actually gives an increased amount of loss of sucrose progressively from affination syrup, first crop, and second crop. The invert produced was lower progressively, showing that combination had taken place. The use of symbols is misleading; C.V. is used for crystallization velocity without explanation, whereas C.V. is already used for the coefficient of variation. A few spelling mistakes occur, including, p. 193, "fosfation". These are poor examples of an otherwise very good book. It is one that should be on the shelves in all sugar factories, refineries and head offices, and should be utilized for new work as well as for judging everyday work.

H. C. S. DE WHALLEY

Les Modèles du Noyau

Par Michel Trocheris. (Bibliothèque des Sciences et Techniques Nucléaires.) Pp. 152. (Saclay: Institut National des Sciences et Techniques Nucléaires; Paris: Presses Universitaires de France, 1959.) 26 N.F.

THE nineteen-fifties have been marked by a vigorous growth in the theory of nuclear structure and the models used to represent it. This book originated in a series of lectures delivered at Saclay in 1955, and the value of its publication nearly five years later might seem questionable. The subject-matter of this book, however, is by no means as general as its title might suggest; it is concerned with an elementary introduction to some of the techniques used in calculations based on the models considered (the Fermi gas, the shell model and the Bohr–Mottelson collective model), rather than the physics of the models themselves or their detailed application to experimental data. Such techniques are often less time-dependent than the models which suggest them, but the meagreness of physical discussion must inevitably limit the usefulness of the book. A second limitation is the almost complete omission of nuclear reaction and decay properties from the discussion. Such properties are as important to present-day nuclear models as the spectra and static moments.

Within these limits the author has written a clear, elementary account of the subject, and this book may form a useful bridge between an undergraduate course of quantum mechanics and the specialized monographs on the various nuclear models.

J. M. SOPER

Nutzenergie aus Atomkernen

Von Dr. Karl Rudolf Schmidt. (Einführung in Physik und Technik von Kernreaktor und Atomkraftwerk, Band 1.) Mit Beiträgen von Hans Günter Heitmann, B. Logermann und Wolfgang Mayer. Pp. xvi+594. (Berlin: Walter de Gruyter und Co., 1959.) 106 D.M.

OF the many books currently available on reactor science and engineering, this must surely be the most ambitious in scope, dealing as it does with almost all aspects of the subject. Starting with elementary atomic and nuclear physics, it leads on to radiation and radioactivity, particle accelerators, nuclear stability and the chain reaction. This in turn leads to elementary neutron physics, reactor theory and lattice calculations.

To note that the subject of nuclear forces, for example, is dealt with in about half a page is not to criticize the book, since its aim is clearly to serve largely as an introduction to the physics side of the title, while providing a more complete analysis of the engineering and technological side. This latter, which takes up more than half the book, deals very thoroughly with such diverse aspects as chemical separation, ion exchange methods, trans-plutonium elements, thermodynamics (including turbine theory, heat transfer, heat exchangers, etc.) and a long section on the properties of materials of interest to reactor designers.

The text is very well supplemented by tables, graphs, references and even nomograms, but, surprisingly, has no index, while topics like reactor kinetics, fission-product poisoning, and shielding are apparently examined in Volume 2, rather than in the physics part of Volume 1, and reactor stability is ignored entirely.

K. R. E. SMITH

THE IAN CLUNIES ROSS ANIMAL RESEARCH LABORATORY, PROSPECT, NEW SOUTH WALES

By Dr. IAN W. McDONALD

Chief, Division of Animal Physiology, Commonwealth Scientific and Industrial Research Organization, Australia

THE dominant role of the sheep industry in Australia's economy has naturally influenced the trends of agricultural research. During the early phase of research sponsored by the State and Commonwealth Governments, attention was focused primarily on the control, treatment or prevention of the numerous diseases which impaired the productivity of flocks. As effective procedures were steadily acquired, it became clear that there was scope for equally useful research on the productivity of sheep. Plans for such a development had gradually been matured by the late Sir Ian Clunies Ross (chairman of the Commonwealth Scientific and Industrial Research Organization), Dr. L. B. Bull (then chief of the Organization's Division of Animal Health and Production) and Mr. H. B. Carter (now with the Animal Breeding Research Organization of the Agricultural Research Council in Britain). The funds to initiate the project were made available by the Australian Commonwealth Government through the Wool Use Promotion Act of 1945 and the Wool Industry Fund Act of 1946. More recently, additional financial aid has been provided directly from the sheep industry by a levy on wool.

A site of 116 acres was acquired at Prospect, a village twenty miles from Sydney, where some of the earliest farms in the first Australian colony were established. The Laboratory, originally called the Sheep Biology Laboratory[1], was gradually developed over the years 1953–59, and was staffed by research groups which had been temporarily accommodated in sister laboratories. The main laboratory block, shown in Fig. 1, was completed in 1959 and officially opened on March 9 by Lord Casey, who until his recent elevation to the peerage had been the Minister-in-Charge of the Commonwealth Scientific and Industrial Research Organization.

As a tribute and a memorial to Sir Ian Clunies Ross, who had contributed so much to the sheep and wool industry through his own research as well as his official direction of the research organization, the Laboratory was renamed the "Ian Clunies Ross Animal Research Laboratory".

The Laboratory was initially directed, on a part-time basis, by Dr. C. W. Emmens, professor of veterinary physiology in the University of Sydney. In 1955, I was appointed officer-in-charge, and an assistant to the chief of the Division of Animal Health and Production, Mr. D. A. Gill. On the retirement of Mr. Gill in 1959 this Division was reorganized and I was appointed chief of the new Division of Animal Physiology, of which the Clunies Ross Laboratory plays the major part.

The present staff includes 42 graduates and 56 technical assistants. The main building contains 24 laboratories of modern design with full service facilities and air conditioning, a comprehensive library, conference room and administrative offices; the floor area is about 25,000 sq. ft., and the cost of construction was £A.240,000. Two large animal houses accommodate 300 sheep in single pens, while another 700 sheep are run out-of-doors. The usual small laboratory animals, used primarily for bio-assays, are housed in fully air-conditioned rooms. A

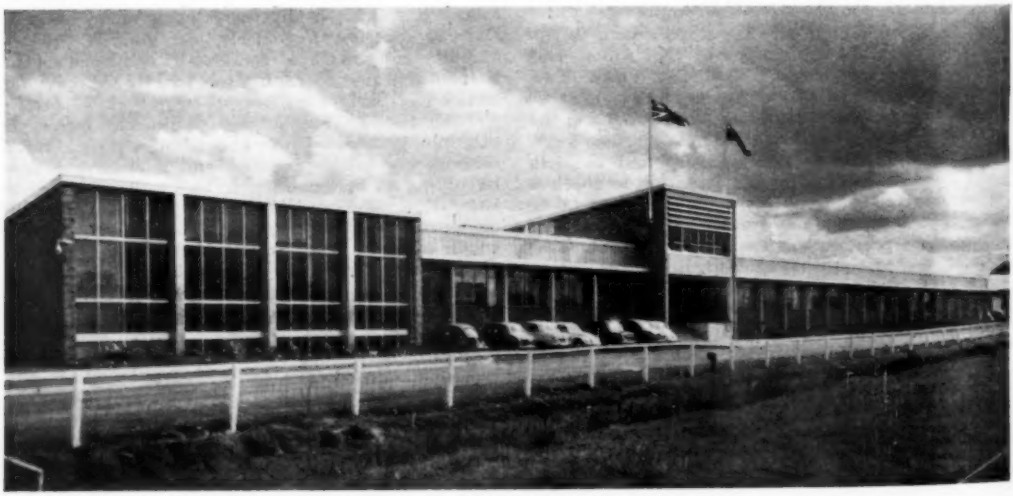

Fig. 1. The Ian Clunies Ross Animal Research Laboratory

modern operating theatre caters for experimental surgery. A number of small fields with sown pasture provide for 'pilot scale' studies on the grazing animal. A well-equipped workshop constructs and maintains much of the special equipment required.

The use of radioactive isotopes in animals as large as the sheep poses some special problems in protection of personnel and disposal of wastes. To meet these problems, an isolated block is being built with accommodation for experimental animals and specially fitted laboratories for handling highly radioactive preparations and for initial processing of biological samples. Laboratory equipment has been provided for preparative and analytical work. Amino-acids labelled with sulphur-35 have proved especially valuable in work on wool growth, and chromium-51 has found application as a marker in studies on digestion. Tritium, carbon-14, iodine-131 and phosphorus-32 have also been used in familiar biological techniques.

The work of the Laboratory falls into seven main categories: reproduction, endocrinology, intermediary metabolism, dermology (morphogenesis, structure and function of the skin), bioclimatology, nutrition, and ecology. The objective has been to expand knowledge of the special physiology of the sheep, with emphasis on those aspects which are likely to lead to more efficient productivity of healthy animals. Sheep are reared under the most diverse conditions in Australia—from the semi-arid, tropical regions of central Queensland, through the temperate grasslands of New South Wales, the tablelands of the Great Dividing Range, to the cool temperate, high-rainfall zones in Tasmania; from pastures so sparse that 10 acres may be allotted to each sheep, to rich highly developed pastures carrying several sheep per acre. With such diversity, it is difficult to generalize on the topic of animal husbandry. As a consequence, the research programme has been designed to provide a sound physiological basis on which husbandry procedures, appropriate to each of the many special regions, can gradually be developed. Some illustrations from recent work are given in the following paragraphs.

It has been known for some time that exposure of rams to high ambient temperatures may lead to seminal degeneration. The mechanisms regulating testicular temperature show very interesting features. The long, coiled, spermatic artery is in intimate contact with a venous plexus, forming a complex which greatly reduces pulse pressure in the artery, and which acts as a highly efficient heat-exchanger. The large temperature gradient between aortic blood and testicular tissue (some 5–6° C.) appears to be derived from scrotal heat loss, and the arterio–venous relations permit the afferent arterial blood to respond quickly to changes in scrotal temperature. It is probable that the efficiency of the scrotum as a heat-dissipating structure is a major factor determining the fertility of the ram in periods of high environmental temperature.

Fertility of ewes has been examined from several angles. The well-defined sexual season of breeds developed in high latitudes is not found in Merino and cross-bred sheep, many of which do not show a definite anœstrus period. The British breeds enter their breeding season in response to declining hours of daylight; but this cannot explain the activity of Merino ewes which may enter their breeding season in the spring with increasing hours of daylight. Clearly other factors, including ambient temperature, must be involved. Climate also influences the frequency of twin ovulations in the ewe. Experiments are being conducted under controlled conditions of light and temperature in the Laboratory to unravel these environmental factors and to determine the physiological pathways by which ovarian function and sexual activity are influenced.

The occurrence of œstrogens in pasture plants was first observed some years ago in Western Australia and has recently been observed in eastern States. This can lead to infertility, dystokia or neonatal deaths to a serious degree, and attempts are therefore being made to develop bioassay techniques as a preliminary to further investigation of the problem. Ovariectomized ewes and wethers appear to be promising material for this study.

Artificial insemination in bovines has been developed, in many countries, to a highly successful, routine procedure; in the sheep, however, the technique has proved less satisfactory. One achievement required for successful application is the induction of œstrus and ovulation at a pre-determined time; procedures for this are being developed; but for reasons not yet clear, fertility has remained rather low. The processes of sperm transport, fertilization and implantation are being examined to throw light on possible interference with fertility.

The metabolism of the pregnant ewe has been the subject of extended study. It has been shown that a number of factors contribute to make the ewe highly subject to nutritional and environmental stresses in late pregnancy, terminating in death of the ewe with pregnancy toxæmia or the birth of weak lambs with high neonatal mortality. The limited supplies of glucose available for absorption from the gut, the preponderance of volatile fatty acids as sources of energy, and the efficiency of the fœtal lamb in withdrawing maternal blood glucose, all predispose to a physiological hypoglycæmia; this, in turn, leads to hepatic inadequacy and adrenal hypertrophy, which permits, under appropriate stress, great increases in circulating corticosteroids, the development of a diabetic state, and profound interference with the central nervous system. Only the outlines of these metabolic inter-relationships have yet been established—much remains to be learnt of the factors governing appetite, digestion in the pregnant ewe, gross energy transactions, the major metabolic pathways, the demands of the fœtus for glucose and amino-acids, the capacity of sheep tissues for gluconeogenesis, the endocrine control of metabolism and the sequence of events leading to metabolic failure.

The new-born lamb arrives into an environment that is all too frequently hostile; it is perhaps scarcely credible that, even in the tropics, lambs may die from cold stress. There is a formidable list of infective diseases which account for much lamb mortality, but many controlled observations have shown unequivocally that, in Australia, most deaths in new-born lambs can be attributed to exposure and/or inadequate nutrition. This has stimulated interest in the physiological mechanisms that enable the fœtal lamb to cope with its sudden change in environment, the influence of maternal nutrition on fœtal development, and the initiation of lactation in the ewe.

The physiological mechanisms governing wool growth have proved to be highly complex. The wool follicles are all formed in the fœtal lamb, though not all are producing fibres at the time of birth. Even

during fœtal life, the development of follicles can be influenced by maternal malnutrition, with permanent detrimental effects. Maturation of follicles occurs rapidly in the lamb, and with increasing age, the follicles become progressively less susceptible to irreversible damage. The three major fields of interest regarding wool growth are endocrine control, effects of nutritional status and climatic influence. The anterior pituitary gland has the major controlling influence, but the individual hormones responsible have not yet been determined; this work has led to an intensive study of the fractionation of pituitary proteins, which has shown the multiplicity of proteins present in the most highly purified preparations at present available. The adrenal corticosteroids have a strong inhibitory effect on wool growth, while administration of thyroxine leads to enhancement of wool growth. There appears to be no specific effect of the sex hormones on wool growth.

Climatic influence on wool growth is definite, with increase during the summer months and decrease during winter. The relative contributions of day-length, ambient temperature or other environmental factors are being studied experimentally with animals maintained in artificially controlled climates. Concurrently, the seasonal variation in reproductive performance can be studied with the same experimental animals.

Wool growth-rate is very sensitive to the nutritional status of the animal. The fact that wool is essentially a protein 'secretion' led to the justified interpretation of earlier work that wool growth was a function of protein intake. More recent work has shown the curious fact that, provided the protein content of the ration is above a certain minimum level, energy intake dominates the growth-rate. The physiological mechanisms involved here remain to be elucidated. Under natural grazing conditions, the influences of nutrition and climate on wool growth are confounded, and much ecological study is needed to clarify the situation.

Biochemical work on wool growth has been greatly assisted by the use of amino-acids labelled with sulphur-35 or carbon-14. The extremely rapid incorporation of labelled cystine into the growing wool fibre (within 1 min. after an intravenous injection) has also provided a tool for the precise measurement of rate of growth in length of fibres during short time intervals, and for critical examinations of differential changes in length and diameter within individual fibres.

Nutritional research has been aided by the construction of two respiration chambers. The closed-circuit design has been selected, with automatically controlled conditioning equipment for varying the chamber environment over a wide range and in constant or cyclic conditions. Experimental sheep are housed and trained in an air-conditioned room so that seasonal influences are reduced to a minimum.

For research in bioclimatology, two climate rooms have been constructed, each capable of accommodating fourteen sheep. In one room a wind tunnel has been built, to enable experimental animals or mechanical models to be exposed to controlled conditions of temperature, humidity, wind and simulated rainfall. Apart from studies mentioned above on wool growth and reproduction, the major projects concern the physiological mechanisms involved in cold stress and heat stress.

The Clunies Ross Laboratory is still in a formative stage; but already it is clear that the application of the biological sciences (physiology, biochemistry, histology, ecology) can throw new light on one of the most ancient of man's vocations—the husbandry of sheep.

[1] Emmens, C. W., *Nature*, **171**, 597 (1953).

RECENT SCIENTIFIC ACTIVITIES AT HALLEY BAY: INTERNATIONAL GEOPHYSICAL CO-OPERATION, 1959

By M. J. BLACKWELL*

Introduction

ON January 10, 1959, a twelve-man party of the Falkland Islands Dependencies Survey arrived at Halley Bay to take over the station from the Royal Society's International Geophysical Year expedition[1]. Following the last-minute decision to continue the scientific programme during the International Geophysical Co-operation, a relief team had been hurriedly organized of personnei seconded from outside bodies, including five scientists from the British and South African meteorological services and five technicians from the Services.

The Falkland Islands party had three links with earlier teams: Lieut. G. R. Lush, R.N. (leader) and D. W. S. Limbert had been members of the 1956 advance party, while J. A. Smith remained from the 1958 party. None of the team, however, was able to spend more than a week or two in preparation for polar work; each was entirely dependent on experience gained in his normal professional occupation. An outline of the main activities of the team and a few of the significant results are described below.

* Seconded from the Meteorological Office, Air Ministry, as Chief Scientist, Halley Bay, during the International Geophysical Co-operation.

Surface Meteorology

Full synoptic observations were made every three hours, and instrumental readings were taken every hour. Because of the accumulation of snow, the anemometer tower needed an extension of 15 ft. A ventilated thermocouple psychrometer, developed by D. Tribble of the International Geophysical Year party, was used throughout the period to give the first complete year of accurate humidity data at very low temperatures. It has a potential accuracy of a few hundredths of a degree in ice-bulb depression and this was more closely realized by adding extra

radiation shields. Trials were carried out by D. W. S. Limbert of a phosphorus pentoxide electrolytic hygrometer[2] which is capable of giving a continuous record of absolute humidity below − 20° C. This is the limit below which the conventional hair hygrograph becomes useless[3].

Upper Air Meteorology

The International Geophysical Co-operation programme provided for one ascent daily. One of the immediate problems facing the team was the maintenance of access to the balloon shed, which was already two-thirds buried by drift snow. An annexe, 16 ft. square, was built in front of the main door and covered with hinged flaps, just above the snow-level. The floor of the cavity thus created led down in easy steps to the old entrance. This arrangement, devised by G. R. Lush, made it necessary to launch the 1,250-gm. balloons out in the open. The radio-sondes and wind-finding radar targets had to be attached, on the run, before the balloons were torn to pieces in the frequent blizzards. By using unwinders with trailing aerials, quick-action clips for attaching the rigs and by dogged teamwork, ascents were achieved on all but 50 days; of these, 46 were gale days. It was surprising that balloons which had been twisted into fantastic shapes during the launches would reach heights of well over 20 km. During the winter months the balloons were dipped in a mixture of lubricating oil and kerosene to prevent premature bursts at relatively low heights. Without this treatment, heights of 10 km. would be typical, whereas 20 km. was consistently reached throughout the winter. Preheating of the balloons also contributed to the excellent performances, which are summarized in Table 1.

Table 1. Mean Heights reached in Upper Air Soundings

Month	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Year
Mean ht. (km.)	22·7	23·3	22·5	22·3	19·4	21·3	20·3	21·3	22·4	22·4	22·8	21·9	21·9

In addition to the work of J. A. Smith, who organized the upper air programme, it is a pleasure to record the contributions of our two South African colleagues, G. M. Artz and J. Bothma, who had not previously encountered the British radiosonde or the Decca wind-finding radar equipment.

Other Meteorological Activities

Continuous records were maintained of total and diffuse solar radiation and of total net flux. Additional measurements were made of the short-wave albedo of the ice shelf. A new hut was built for the ozone spectrophotometer. This was mounted on sledge runners to facilitate regular recovery from snow drifts. By sighting the instrument through a small hatch in the roof, observations could be made in all weathers. On one occasion, zenith sky measurements were made successfully in a wind of 55 knots with gusts to more than 70 knots. The development of sledge-based huts for similar scientific activities is one which shows considerable promise for the future. The normal summer observations were linked by Moon observations during the polar night. Six series of *umkehr* measurements were made to investigate variations in the vertical distribution of ozone. It had been observed in previous years[4] that the spring increase in total ozone content was quite sharp at Halley Bay. It is interesting to note that this increase was only gradual in 1959. The discovery of new modes of atmospheric behaviour gives some measure of reward to those responsible for urging the extension of an observational programme in a remote part of the world. Other features in which 1959 differed from previous years were noticeable in both surface and upper air climatic sequences.

Recording of surface ozone was continued using a new electrolytic detector element designed by Dr. A. W. Brewer, of the Clarendon Laboratory, Oxford. It replaced the transmogrifier system[5] previously in use. There appeared to be good continuity between the two systems, the change-over taking place in March. Monthly means reached a clear maximum in winter and minimum in summer. In 1958, a secondary decrease which occurred at mid-winter[4] is attributed to the concentration of local contaminants beneath intense surface inversions. The 1959 apparatus was installed at the new site for the spectrophotometer, well clear of smoke from the main living hut. This work was carried out by J. A. Smith.

Another meteorological project, attempted by D. W. S. Limbert, was the investigation of the significant parameters involved in the growth-rate of sea ice. Temperature profiles, in air and ice, and wind profiles were obtained together with density and salinity samples. Combined with other data on energy balance (for example, radiation fluxes) available from base records, these measurements should help to assess the rival merits of recent theoretical predictions.

Movement of the Ice Shelf

The most significant work of the year was the determination of the movement of the ice shelf, both by conventional and by less usual methods. Owing to the scatter in astronomical fixes caused by intense refraction, it has taken three years to establish the movement within reasonable limits. A provisional estimate, based on results for 1957 and 1959 only, gives a W.N.W. movement of about 600 yd. a year. Simultaneous azimuth observations made during the same period show an anti-clockwise twist of the ice shelf. The best fit of the available results gives annual rates of twist of about 18 min. of arc in 1957, diminishing gradually to 15 min. in 1959. The 1959 work was carried out by M. A. Sheret and myself in conjunction with the geomagnetic programme.

We also repeated the local magnetic survey made a year earlier by A. Blackie and J. MacDowall[7], of the International Geophysical Year party. This had shown a vertical force anomaly of several hundred gamma to the north-west of the base and close to the ice front. It was wondered whether movement of the ice shelf over such an anomaly could be detected as little as a year later. The pattern of eight stations covering an area of some 16 sq. km. was re-surveyed, again using a *B.M.Z.* magnetometer. The contours of the anomaly were fully confirmed, but it was immediately apparent that the ice shelf had moved forward, carrying the stake pattern with it, relative to the underlying anomaly. A fairly precise evaluation of the movement would require a closer spacing of the grid and a small closed loop in the magnetic contours. Nevertheless, a provisional analysis of the results (made independently of the astronomical

determination) gives a W.N.W. movement of about 500 yd. a year. There is clearly a wide opportunity for the use of this simple and direct technique in areas where the magnetic topography is suitably convoluted. Though the attainable accuracy cannot compete with orthodox survey work, successive surveys could be carried out during brief summer visits.

In addition to the two methods so far described, D. W. S. Limbert fixed the position of two icebergs (which were thought to be grounded on the sea bed) by triangulation from a measured pattern of movement stakes. By repeating the survey after an interval of nine months and using the change in bearings from the icebergs, it was possible to derive a third estimate of the movement of the ice shelf. The provisional reduction of the results gives a westward movement of about 400 yd. a year. Simultaneous with the absolute translation and twist of the shelf, the pattern of movement stakes was observed to undergo deformation, with relative changes of 1 or 2 ft. in 1,000 yd. occurring within a year.

Glaciology

Measurements of snow accumulation were made against three different patterns of stakes: the standard box pattern for net monthly accumulation; a line pattern, nearer to base, for day-to-day ablation and accumulation; and two units (resembling goal posts) for studying the migration of sastrugi. It was noticeable that aggregate totals of accumulation from a given pattern of stakes would sometimes exceed and sometimes fall short of the corresponding totals from the other patterns. The differences were maintained for perhaps months at a time, and are thought to show evidence of the long-term migration of shallow, large-scale features on the ice shelf. Measurements of density and sub-surface temperature profiles were also made. The work was carried out by D. W. S. Limbert.

Other Geophysical Activities

The geomagnetic programme was maintained by M. A. Sheret and myself. Because of the continual rise of the snow surface, the fixed mark could no longer be seen from the declinometer in the magnetic hut. The problem was solved by building a new declinometer room above the north porch which could be entered from below through a small hatch. The fixed mark could then be viewed above the snow-level. To ensure continuity of reference azimuth the angular difference between the old and new sites was determined by theodolite from the mark. Settling of the foundations of the magnetographs required regular adjustments to be made to the variometers but caused no undue difficulty.

A further aspect of the movement of the ice shelf is that the annual means of the magnetic elements, which normally yield the secular variation of the Earth's field, will include an additional term due to the movement of the magnetic hut relative to the magnetic topography of the sea bed. This spurious component can be allowed for when the movement and the contours of the isomagnetics are both accurately known. In addition to the local magnetic survey, the opportunity was taken to obtain measurements of vertical force during the course of a 33-mile physiological sledging trip to the east-north-east of the base. This work was done by Dr. J. N. Norman and G. M. Artz. When combined, the two surveys show that a complex system of magnetic contours covers the whole region so far explored. We can be reasonably certain that there is a corresponding complexity in the underlying geological structure, but it now seems improbable that anything can be deduced about the physical contours of the sea bed. Soundings made from the Falkland Islands Dependency Survey relief ship, R.R.S. *John Biscoe*, indicate that the ice shelf is floating in the area of Halley Bay and that their are no significant shallows which might account for the coastal features of the ice front.

The auroral physics programme was continued with quarter-hourly visual observations, and all-sky camera records during significant displays. M. A. Sheret, who carried out the work, is also attempting to investigate auroral–geomagnetic relationships. An investigation of tidal currents off the coast, made by me, was a continuation of a programme sponsored by the National Institute of Oceanography. The potential difference created by the motion of sea water across the Earth's magnetic field was detected by a pair of silver/silver chloride electrodes suspended below the sea ice near the bay[8]. Tidal variations can be distinguished in the records during periods when geomagnetic activity is slight. Seismological records were also continued until the end of the year.

Biology

Two distinct programmes were carried out by Dr. J. N. Norman. A physiological investigation into the micro-climate of individuals was sponsored by the Medical Research Council. Integrated skin temperatures were obtained, by means of vests sensitive to temperature worn next to the skin, for four subjects going about their normal duties. Additional readings were taken during the man-haul sledging trip already referred to, and these were linked with observations on dehydration and energy balance of individuals.

A separate investigation in the field of penguin biology was carried out during the winter months. By building a small satellite base with living hut and mobile generator, planned by G. R. Lush, it was possible to observe the vast Emperor penguin rookery (3 miles to the west of base) from close quarters. As a result, J. N. Norman, helped by M. A. Sheret, was able to collect a timed series of embryos and to extend our knowledge of the breeding cycle of these primitive creatures.

An attempt has been made to describe something of the scope and variety of the work now being carried out by the Falkland Islands Dependencies Survey at Halley Bay, with particular emphasis on the less routine aspects. We are indebted to the International Geophysical Year parties of 1956–58 for the excellent start given to the station. This article is published with the permission of the Director, Falkland Islands Dependencies Survey.

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[2] Brewer, A. W., Air Ministry, London, Met. Res. Pap. 1084.
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SPECIFIC INHIBITORS OF THE ADRENAL CORTEX

By Prof. J. H. U. BROWN

Department of Physiology, Emory University, Atlanta, Georgia

THE endocrine system is composed of many glands in often inaccessible locations with a wealth of interrelationships. In order to investigate the individual hormones it is necessary to remove the gland, or to inhibit in some way its function. Removal is often difficult and in many cases impossible. For this reason, one of the aims of endocrinology has been the development of substances which specifically inhibit a single endocrine organ.

It has been possible for many years to block the action of two of the endocrine glands with specific inhibitors. The use of alloxan to destroy the beta cells of the islets of Langerhans in the pancreas and thus produce true diabetes mellitus has permitted great strides in the study of the action of insulin. The use of the various thiouracils to inhibit synthesis of thyroid hormones and permit investigation of thyroid physiology is too well known to require discussion. The search for specific inhibitors of the other endocrine glands has, in general, been disappointing.

The use of a specific inhibitor for the adrenal cortex would be of special value to the endocrinologist since in most species it is impossible to remove the cortex without simultaneously removing the medulla, and since the presence of accessory glands in all species often makes the operation complicated. Furthermore, a substance which would inhibit specific pathways of steroid synthesis would be of inestimable value in elucidating the synthesis of the various steroids.

Amphenone *B*

Great interest was aroused in 1950 when Hertz, Allen and Tullner[31] reported that a new compound, *p*-aminopropiophenone (amphenone *B*) (Fig. 1), synthesized by Allen and Corwin[1] appeared to cause enlargement of the adrenal and thyroids of rats. Two explanations were possible: the pituitary could be directly stimulated to increase output of its hormones and thus stimulate the target organ to greater function, or secondly, the target organ itself might be affected by the drug. Administration of adrenal or thyroid hormones with the amphenone completely prevented the enlargement of the glands and indicated the site of action to be at the end-organ level. The results also suggested inhibition rather than stimulation of the adrenal and thyroid and demonstrated that the enlargement was due to compensatory hypertrophy which usually occurs in the absence of the target organ hormone consequent to the increased output of trophic hormones of the pituitary[35].

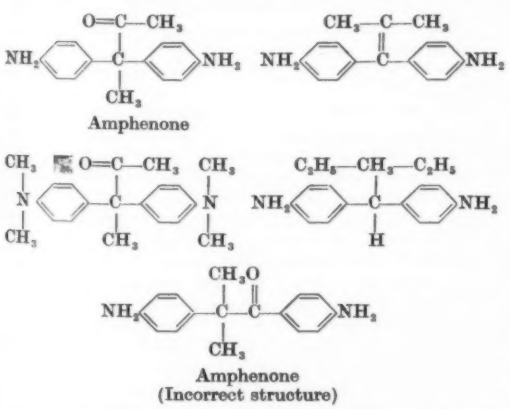


Fig. 1. The true and incorrect structure of amphenone and the various closely related substances which possess adrenal inhibitory action

The results were soon confirmed by Chappel *et al.*[13], by Coste *et al.*[15] and by Danowski[16], who reported finding increased numbers of basophils in the pituitary, increased lipid deposits in the adrenals, loss of colloid and increased epithelial cell height of the thyroid cells, and fatty infiltration of the liver of rats treated with amphenone. The results taken as a whole indicated decreased function of the thyroid and adrenal with increased output of pituitary trophic hormone.

The picture was apparently clear-cut until Hogness[26], Heming[30] and Vogt[51] presented results suggesting stimulation rather than inhibition of the thyroids and adrenals as a result of administration of amphenone. Vogt cannulated the adrenal vein and demonstrated increased steroid production when amphenone was given to rats. In the light of later experiments on both man and other animals, these results must be interpreted as due to peculiarities of dosage, to strain differences, or to unknown factors.

Despite the contradictory information, other experiments sustained the interest in amphenone *B*. For example, several workers[49,51,65] demonstrated that amphenone inhibited the production of aldosterone by the adrenal cortex at a much lower dosage than that required to suppress the output of the other adrenal steroids.

In view of the promise of amphenone as an adrenal cortical inhibitor attention was turned to the production of derivatives which might prove to be more effective or less toxic. Hertz[26] found that of some 25 derivatives only 3 had promise as inhibitors (Fig. 1). It should be emphasized that the structures reported by Allen and Corwin[1] and utilized by Hertz were not correct. In 1957, Bencze[16] found that the molecule became re-arranged (Fig. 1), so it is possible that other derivatives might prove more satisfactory theoretically than those based on the old structure.

Investigation was also proceeding on the mode of action of amphenone. Marks[50] reported that the drug appeared to inhibit pathways of steroid metabolism which did not require oxygen, that is, glands treated with amphenone to complete inhibition of steroid synthesis still utilized the same or greater amounts of oxygen. Marks also found that cholesterol accumulated in the adrenals of rats treated with

amphenone and he suggested that the site of inhibition might be at the level of conversion of cholesterol to the precursors of the adrenal steroids (pregnenolone, for example). Rosenfeld and Bascom[66], in a much more elaborate study, utilized the newer methods of steroid identification and found that amphenone inhibited the 11, 17 and 21-hydroxylation of pregnonolone and probably inhibited the conversion of the 3-hydroxyl to a 3-ketone.

The demonstration of the action of amphenone in animals led to its immediate use in human patients suffering from adrenal cancer or hyperadrenalism. The drug was tried with several patients having adrenal carcinoma[25,34,33] with successful results. In these patients there was a decreased excretion of 17-ketosteroids, some evidence of tumour regression, and a depression in the excretion of 17-hydroxycorticoids. The experimental results had shown evidence of thyroid blockade as well as an adrenal action and a series of papers[34,69,73] demonstrated that iodine-uptake was depressed, organic binding of iodine was markedly decreased, and that enlargement of the thyroid occurred in man treated with amphenone. The drug was also tried in patients with hyperadrenalism[43,53,73,76] with considerable success as measured by regression of symptomatology.

However, difficulties soon arose in the use of amphenone. Patients developed thyroid blockade, toxic symptoms including blood dyscrasias, liver damage and general malaise. A major symptom was extreme drowsiness[34], which could be predicted from the anæsthestic property of the drug previously reported in rats[32]. It soon became obvious that the many disadvantages might outweigh the advantages of the drug.

DDD

At about the same time as the synthesis of amphenone, Nelson and Woodard[54] were testing the toxicity of insecticides, including the derivatives of diphenylethane. They reported that high doses of the material 1,1-dichloroethane, 2,2-*bis* parachlorophenyl (DDD) (Fig. 2) produced necrosis of the adrenal cortex in the dog. By contrast, DDT, the tri-chloroethane, was totally ineffective.

The compound was immediately taken up by Nichols and his co-workers[56–62], who found in short order that dogs fed DDD became insulin-sensitive, that the usual biphasic response of the blood sugar to alloxan did not occur in dogs treated with DDD, and that these animals did not respond to adrenocorticotrophic hormone injections with the usual increases in uric acid or allontoin excretion. Since all these responses are usually attributed to the metabolic activity of the glucocorticoids of the adrenal cortex an immediate presumption was made that DDD either suppressed the activity of the pituitary or of the adrenal cortex. Nichols[57] at first postulated that DDD blocked the effect of adrenocorticotrophic hormone on the adrenal, but was soon forced to the conclusion that DDD acted directly on the cortex.

Other evidence of the inhibition of the adrenal cortex was obtained by Little[48], who found that dogs fed DDD developed a depression of glomerular filtration-rate similar to that of adrenalectomized dogs, and by Nichols, Kaye and Larson[62], who found that DDD potentiated the action of barbiturates.

The first direct proof of the adrenal suppressing activity of the substance was obtained by Brown *et al.*[9,10], who found that the excretion of 17-hydroxy-

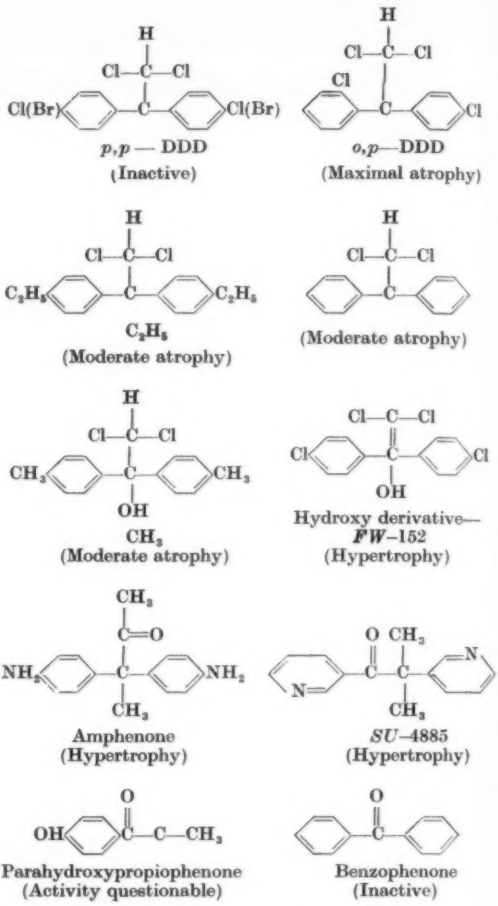


Fig. 2. Structures related to DDD with some indication of the activity as adrenal cortical inhibitors

corticoids was markedly depressed in dogs given DDD. They also reported that the effect could not be reversed by the administration of adrenocorticotrophic hormone, indicating the adrenal site of action, but that the effect could be completely reversed by the administration of cortisone. The results were soon confirmed by Kuchmeister *et al.*[43] and by Vilar and Tullner[79].

The action of DDD in the dog was clear. Identical results were obtained in cattle[50] and in the domestic fowl[55]. Unfortunately, the results were not so clear in the rat. Brown[6,7] found that DDD had some influence (decrease) on the eosinophil response of the rat to adrenocorticotrophic hormone and that it may have alleviated the course of alloxan diabetes in the same animal. Verne and Wegmann[77,78] claimed that DDD produced adrenal cortical atrophy in the rat. This was in direct contradiction to Brown[6] and others, who reported hyperplasia of the adrenal in DDD treated rats. Stoner[71], Haag *et al.*[27] and D'Arcy[19] failed to find evidence of any effect of DDD on this animal. It would appear that the situation is unresolved, and that further work is necessary. Perhaps the isomers to be discussed below will provide a compound active on the rat adrenal. This is a problem

which must be attacked since the rat is the most commonly used laboratory animal in the investigation of adrenal function. It may be an interesting coincidence that DDD appears to be most active in those species which secrete hydrocortisone as the major metabolic steroid and is inactive in the one animal tested to date (the rat) which does not secrete this steroid.

Because of the possible use of DDD as an adrenal blocking agent, many derivatives have been investigated (Fig. 2). The most active of these is 'Perthane', the di-ethyl derivative of DDD. The drug is an active adrenocorticolytic agent, but appears to be active only at about three times the dose required for DDD[3,4,14]. The relationship between structure and function is somewhat obscure. Many derivatives have some activity and result in atrophy of the cortex, but there are several in the series which appear to produce hyperplasia of the adrenal[14,44]. It is not known if these later compounds act in a manner similar to that of amphenone, which they closely resemble in structure.

It is known that the action of DDD and amphenone on the adrenal cortex are not similar. Nichols and Hennigar[40] fed amphenone to animals previously treated with DDD, and DDD to animals previously treated with amphenone. They found that amphenone could not prevent the atrophy from DDD, but that the usual hypertrophy due to amphenone could be entirely prevented by DDD.

In addition to the more direct measurements of adrenal function under the influence of DDD (steroid excretion, etc.) there are more subtle indications that the gluco-corticoids are either not being produced or not active at the tissue-level. Candela and Candela[11], for example, found that the diaphragms of DDD-treated rats were insulin-sensitive when compared to normal diaphragms, while Nichols, Davis and Green[56] found that the adrenal cortices of dogs treated with DDD did not respond to adrenocorticotrophic hormone with the usual increase in oxygen consumption found with normal glands. Naidoff *et al.*[53] found that DDD lowered the blood-sugar of diabetic rabbits, and Tornblom[74] reported that DDD lowered the serum polysaccharides of the same species. All these observations suggest responses of the peripheral tissues similar to those obtained in adrenalectomy.

In course of investigations on DDD, many workers had noted that the dosage required to produce a given response appeared to vary from batch to batch of the technical material[8]. Conflicting reports were obtained from laboratories using different batches of DDD. These difficulties were resolved in 1958 when Cueto and Brown[16,17] reported the isolation from the technical material of an active agent which gave consistent responses at much lower dose-levels than that reported for the crude DDD. Characterization of the pure substance led to the conclusion that it was present to the extent of about 10 per cent in the technical products and was the *o,p*-isomer of DDD. The remainder of the technical material was largely *p,p*-isomer.

It was soon demonstrated that the *p,p*-isomer was inactive and that the *o,p*-isomer contained all the activity attributed to DDD. This explained many inconsistencies. Several groups had recrystallized DDD before using it and this effectively removed the active component. Others had used the technical material, and as the active isomer varied from batch to batch, inconsistencies were reported. At about the same time as the isolation of the active agent, Nichols[61] found that an oil isolated from the technical material was active in producing atrophy of the adrenals, but he failed to characterize the material. It was probably the *o,p*-isomer. Vilar and Tullner[79], however, used crystalline *o,p*-DDD and confirmed the results completely.

With the availability of a pure product, it became possible to test the material in man. 'Perthane' had been previously tested[64,72], but large doses were required and some toxicity developed. Sheehan and Nichols had previously tested DDD in man with negative results[70], but they had used the technical material with unknown amounts of *o,p*-isomer. Zimmerman[82], on the other hand, also used technical material and found some tumour regression occurred in cancer patients and that a modest decrease in blood corticosteroids could be elicited with the drug. Brown *et al.*[9] found a definite decrease in blood corticoids in three cancer patients treated with the *o,p*-isomer and Bergenstal, Lipsett, Moy and Hertz[2] found definite suppression of adrenal function in twelve patients with adrenal carcinoma with regression of tumours and objective improvement of the cancer. Some toxicity was reported. Tornblom[75] found that DDD decreased the incidence of hyaline vascular disease in diabetic man and noted that diabetics given the drug required a smaller insulin maintenance dose than before treatment.

To the present very little attempt has been made to determine the site of action of DDD on the adrenal cortex. Large doses of the drug cause necrosis with a complete disappearance of cell structure, but it is known that small doses will inhibit steroid production before signs of necrosis develops. Nichols was able to demonstrate an effect of DDD on the oxygen consumption of adrenals treated with adrenocorticotrophic hormone[56], but he did not attempt to determine the site at which the inhibition occurred. Because of its value as an adrenocorticolytic agent, further work should be done on this phase of the problem. It is also possible that use of the *o,p*-isomer or of some other derivative might make the compound effective in the rat and increase its value immensely.

SU-4885

In 1958, Chart *et al.*[13] reported that a new analogue of amphenone, designated as *SU*-4885 (Fig. 2), was effective in suppressing the adrenal function of dogs, rats and guinea pigs. The drug produced an increase in adrenal-weight, increased insulin sensitivity of experimental animals, had no effect on the thyroid, and was effective in suppressing steroid synthesis in adrenal slices.

Jenkins, Meakin, Nelson and Thorn[40] tried *SU*-4885 in man and obtained a decrease of 90 per cent in steroid excretion. The precursors of the 11-hydroxylated steroids appeared in the urine, indicating blockade of this step. The blockade was confirmed by the finding of large amounts of tetrahydro *E* and *F*. Similar results were reported almost at once by Liddle *et al.*[47]. In a later paper, Jenkins *et al.*[41] again reported almost total suppression of glucosteroid production by the adrenal under *SU*-4885 treatment and suggested that the major difference between *SU*-4885 and amphenone was that amphenone was less selective in action.

The drug has not yet been investigated to the extent which will allow prediction of future use in clinical medicine.

Other Substances

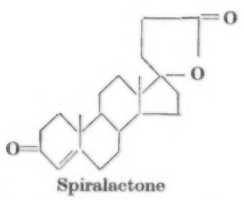
Several other materials have been reported to exert an inhibitory or lytic effect on the adrenal cortex. Dimethylaminoantipyrine and some of its analogues have been reported by Borth[5] to inhibit steroid output of the cortex. The effect is slight and the large doses required do not permit routine use.

Edgren *et al.*[20] found that 6-methyl-17-acetoxyprogesterone decreased adrenal size and function as measured by the 'cold' test. This is inconclusive evidence and no further work has been reported.

Inhibition of aldosterone secretion upon administration of delta-4-cholesterone has been reported by Frederickson[23] and Golden and Overman[26] reported necrosis of the adrenal cortex following infection with *C. diphtheria* in monkeys. The compound para-hydroxypropiophenone (PHP), which is essentially half the amphenone molecule (Fig. 2), has been tested by several groups. Foglia[22] reported that PHP caused a decrease in adrenal weights in rats, Sachsse[27] found improvement of diabetes upon treatment with PHP which might be attributed to decreased adrenal function and a consequent decrease in gluconeogenesis, while Danowski[18] found that the drug decreased adrenal size. In all cases, the effects were not marked and high doses were required.

Steroidal Lactones

A good deal of attention has been directed to a class of drugs which antagonize aldosterone and deoxycorticosterone (Fig. 3). The drug has been extensively investigated by Kagawa[32,34]. The effects are not mediated through the adrenal, and so this drug cannot be classed with those which have a direct adrenal action.



Spiralactone

Fig. 3. The structure of a steroidal lactone with effects on steroid excretion

Structure-function Relationships

All the compounds which are potent adrenal inhibitors appear to possess two six-membered rings linked together by one or two carbon chains. In the case of amphenone and of DDD phenyl groups are attached to the same carbon atom, while in the case of *SU*-4885, they are attached to adjacent carbon atoms.

The effect of substitution on function is not clear. The presence of substitutions on the phenyl rings is not necessary for activity (Fig. 2)[44], but substitution appears to enhance the activity in some cases. The addition of ethyl, methyl, chlorine and bromine to the phenyl group produces a more active compound. The use of nitro, methoxy, or aldehyde substitutions produces a completely inactive compound[4]. The effect of location of the substitution has not been extensively investigated. It is known that the *p,p*-chloro derivative is completely inactive while the *o,p*-chloro derivative is active in a high degree. Compounds are now being synthesized with various steric configurations to investigate this problem.

The substitution on the carbon adjacent to the phenyl attachments is important. A tri-chloro substitution (DDT) is completely ineffective, as is the mono-chloro compound[44]. Removal of the chlorines from the adjacent carbon, substitution of a hydroxyl group on the phenyl carbon, and apparently the presence of a ketone on one of the chain carbons results in hypertrophy rather than atrophy, although a greater or lesser inhibition of action may occur. Apparently more than one carbon must be present in the chain since benzophenone is inactive[72]. In this connexion it is interesting to note that a compound of related structure (3,5-dioxo-1,2-diphenyl, 4-*n*-butyl pyrazolidine) has been reported to have slight adrenal inhibitory activity[5].

It is interesting to note that all the compounds which have inhibiting properties appear to have a six-membered ring, but there appears to be no requirement that it be benzenoid in character. The nitrogen-containing ring of *SU*-4885 appears to be quite effective. Although the active amphenones mentioned by Hertz[36] all contained *p,p*-amino groups on the benzene rings, it is not known whether these are essential to activity. Reasoning from the information available on DDD one could conclude that other substitutions might be equally or more effective.

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OBITUARIES

Dr. W. Lawrence Balls, C.M.G., C.B.E., F.R.S.

DR. LAWRENCE BALLS, who died on July 18, was the foremost of many workers who have been engaged on the development of Egyptian cotton during the present century. His personal investigations have been of great practical importance as well as of outstanding scientific interest. In addition to providing scientific knowledge, he markedly influenced the administrative procedures in Egypt for making that knowledge effective.

William Lawrence Balls was born in 1882. After being educated at King Edward VI School, Norwich, and St. John's College, Cambridge (of which he was a Fellow from 1909 until 1913), he became botanist to the Khedivial Agricultural Society in 1904 and served that body and (later) the Egyptian Department (later Ministry) of Agriculture until his retirement in 1947, except for a period 1915–26, when he was designer and head of the Experimental Department of the (then) Fine Cotton Spinners' Association, Ltd., at Bollington, Cheshire.

During the first phase of his career in Egypt, Balls made detailed physiological studies of the growth of the cotton plant and of the mode of development of the fibres produced from the seeds—the raw cotton of commerce. Many of the results of these fundamental investigations are summarized in his now classical works "The Cotton Plant in Egypt" (1912) and "The Development and Properties of Raw Cotton" (1915). He also made some of the earliest critical genetical studies of plant characteristics and features of fibre quality, and rapidly formed the conclusion that mediocrity and degeneration in both yield and quality were inevitable under Egyptian conditions unless genetic variability was minimized and controlled. From this arose his concept of 'pure line' strains. This work led to the development of methods for making available pure-line seed supplies. During his later career in Egypt, as chairman of the Cotton Research Board of the Ministry of Agriculture, Balls gave continuous encouragement to the policy of crossing and re-crossing of the various strains to exploit to the full the latent characteristics inherent in Egyptian cotton and persisting from the original historical medley of impure cottons.

From the start of his career, Balls was actively interested in the physiological requirements of the cotton plant, and this led him to study the water and drainage requirements for optimum yield under Egyptian irrigated conditions, with the results described in "The Yield of a Crop" (1953). In this work he arrived at the valuable, though possibly depressing, conclusion that the improvements in crop yield made over the years, by himself and his co-workers through better pest control, improved manuring, the development of more prolific strains, etc., had only made it possible to counterbalance approximately the progressive losses caused by the water-logging of the soil associated with the increase

in the water-table following the change from the basin type of irrigation to perennial irrigation.

In considering fibre quality, Balls took as his end-point the quality of the spun yarn, and this interest, together with the circumstances of his career, led him into spinning technology. His "Handbook of Spinning Tests for Cotton Growers" (1920) was a pioneer work that needs little amendment to-day. His "Studies of Quality in Cotton" (1928), based on research work done at Bollington and describing notably the 'drafting wave' and the 'Rule' spinning frame, has influenced much of later technological research and development.

Balls was active to the end, and it was a pleasure to delegates at the Jubilee Conference of the Textile Institute (which had awarded him an honorary fellowship in 1943) in May of this year, to hear from the master himself a paper on the "Beginnings of Botany on a Cotton Crop".

Dr. Lawrence Balls was a great man, in the true sense of the words, who made for himself a career and won by his ability international recognition in a little-known and, at that time, little-regarded field of investigation.

D. W. Hill

Prof. J. Brontë Gatenby

Prof. James Brontë Gatenby, the eminent cytologist, died on July 20 while on holiday at Galway, Ireland. Born in 1892 in New Zealand, he developed a keen interest in entomology and this led him to correspond with Prof. E. B. Poulton in Oxford, where he later went as a student and studied under Prof. E. S. Goodrich. His interest in entomology was reflected in many of his later papers. He graduated in 1916 with first-class honours and was appointed lecturer in histology in Prof. (later Sir Charles) Sherrington's Physiology Laboratory. He soon became interested in cytology, particularly in the inclusions of the germ cells of a wide variety of animals, and he published a long series of papers on this subject. In 1918 he was appointed to a senior demyship at Magdalen College, Oxford, and in 1919 lecturer in cytology in the University of London, where he worked with Prof. J. P. Hill. During these years he produced a considerable number of papers on the embryology, oogenesis and spermatogenesis of both invertebrates and vertebrates. His D.Phil. he obtained from Oxford and his D.Sc. from London.

In 1921 he was appointed to the chair of zoology and comparative anatomy in Trinity College, Dublin, a post which he held until 1959, when he was appointed to a research chair of cytology in the same University. During this period in Dublin he attracted students from all over the world, and he was involved in several fierce controversies relating to the Golgi apparatus—the cell inclusion which he studied in considerable detail. These controversies were mainly concerned with Parat's vacuome hypothesis and also with the question of whether or not the Golgi bodies in many cells were staining artefacts.

Since the Second World War, Gatenby took an increasing interest in electron microscopy. He became firmly convinced of the great potentialities of this technique two years ago when he worked for a while at the Argonne National Laboratory in the United States. He was, however, always insistent on linking this work with light microscope studies. This was his second visit to the United States—in 1930 he spent a year at Yale University as a Seessel Fellow. In 1933 he was appointed visiting lecturer to the University of Louvain.

He had a very extensive knowledge of microscopical techniques, and for many years was a joint editor of "The Microtomist's Vade Mecum". He was on the editorial board of the *Journal of the Royal Microscopical Society* and other learned journals, and was made an honorary member of a number of scientific societies.

To his students Gatenby appeared rather fearsome at times, but to keen and interested ones he gave every encouragement and showed much kindness. He would stand for no sham or bluff. He was in many ways an outstanding representative of the old school of classical zoologists. His teaching and published work reflected his respect and affection for some of the great names of British zoology whom he knew well—Goodrich and Lankester in particular. By his death the world of cytology and zoology has lost an eminent man. He is survived by his second wife and four children by his first marriage.

J. N. R. Grainger

Prof. F. A. Jenkins

Prof. Francis A. Jenkins, professor of physics in the University of California, died at his home in Berkeley on August 3, after an illness of two months. He was a graduate of the Universities of Chicago and Toulouse and had been a faculty member at Berkeley for thirty-one years. He had held Guggenheim fellowships at Utrecht, Uppsala and most recently at Oxford, where his presence and activity in the autumn of 1958 are warmly remembered at the Clarendon Laboratory, at Brasenose College, which made him a member, and by many friends and colleagues.

Jenkins's name is perhaps most widely known through the excellent text-book on optics, by Jenkins and White; but it is his research work, covering a wide field of optics and especially spectroscopy, which has established his reputation among physicists, chemists and astronomers everywhere. His chief contributions were in the field of molecular spectroscopy; of the numerous topics only some of the most important can be mentioned here: his thorough study of the CN bands and of perturbation effects in their rotational structure, the investigation of isotope effects and the determination of isotopic mass ratios (Li_2, BO), his work on the intensity distribution in the rotational structure of bands and on the changes of coupling conditions as the result of increasing rate of rotation (NO), the determination of nuclear spins and nuclear statistics from alternating intensities (Se, C^{14}) and the study of Rydberg series in band spectra.

In the field of atomic spectroscopy, his work on the quadratic Zeeman effect in sodium and potassium, of Zeeman effects in forbidden lines and on hyperfine structure and isotope shift is outstanding. He also made valuable contributions to spectroscopic methods, especially in the field of dielectric multi-layers, studying the performance of broad-band interference filters, the dispersion of the phase change in such filters and the ultra-violet reflectivity of multi-layers containing antimonous oxide and lead chloride. It was especially his knowledge and experience in this field from which the spectroscopy group in the Clarendon Laboratory derived great benefit during his stay there.

His most recent interests were exciton absorption spectra and methods of applying electronic computers to the analysis of band spectra. In the latter field he worked in close collaboration with the Departments of Chemistry and of Astronomy in Berkeley.

A feature common to all Prof. Jenkins's publications is their thoroughness and completeness, both in the measurements themselves and in their theoretical evaluation; there was no room for false claims, either in his work or in his personality.

Those who knew personally 'Pan', as he was called, will have lively memories of his warm personality, his human understanding and friendliness, his sense of humour and the breadth of his interests and concerns. Many will also recall his happy home and family, that meant so much to him. He was strongly attached to California, where he had spent most of his life, and he liked the camping holidays for which that State offers so many opportunities. But he also had strong ties with the Old World and felt very much at home in England and other European countries. His presence and contributions will be sadly missed at many spectroscopic conferences.

H. G. KUHN

Prof. Tadeusz Miłobedzki

PROF. TADEUSZ MIŁOBEDZKI, formerly of the Warsaw Polytechnic, died on July 13, 1959. He led a simple, quiet life and worked successfully until old age. He was a man of great intellectual power, a skilful research worker and an excellent teacher.

Miłobedzki was born at Koło upon Warta on June 16, 1873, and graduated at the University of Warsaw in 1897. He took a special interest in organic chemistry, and was a student of Prof. Wagner, a specialist in terpenes. He also worked in Berlin, Leipzig and Graz, and obtained his Ph.D. at the Jagellonian University in Cracow in 1918.

In 1906, when the Russian authorities had allowed the opening of the Polish Technical College, Miłobedzki was appointed a lecturer in inorganic and analytic chemistry there. In 1915 he took an active part in the organization of Polish university education. He was a co-organizer of the Warsaw Polytechnic and the first dean of its Faculty of Chemistry. In 1917 he was appointed an extraordinary professor at the Central School of Agriculture in Warsaw, where he became the first rector. In 1922 he was called to the chair of inorganic chemistry in the University of Poznań, and in 1929, after the death of Prof. J. Zawidzki, he succeeded to the chair of inorganic chemistry at the Warsaw Polytechnic.

For his outstanding work as a scientist, organizer and teacher, he was made an honorary professor of the Central School of Agriculture at Warsaw and of the Jagellonian University.

In 1922 Prof. T. Miłobedzki became an ordinary member of the Poznań Society of the Friends of Sciences, and in 1932 he was elected to the Warsaw Scientific Society. During 1937–39 he took part in the work of the Polish Committee of the International Union of Pure and Applied Chemistry. In 1945 he became a member of the Polish Academy of Science and Arts and, on its foundation, of the Polish Academy of Science.

Prof. Miłobedzki played a prominent part in the development of the Polish Chemical Society. In 1920 he was one of its founders and in 1928 he was elected president. During 1916–17 he was the editor of *Chemik Polski* and during 1935–39 he was the editor of *Roczniki Chemii* (*Annales Societatis Chimicae Polonorum*).

Prof. Miłobedzki and his pupils carried out important investigations on titration analysis. He also worked on phosphorus compounds, using not only the methods of classical inorganic chemistry but also those of physical chemistry, including the Raman spectra and absorption spectrometry. Prof. Miłobedzki also worked out a commonly applied new method for the detection of acid radicals. His books on analytical chemistry, "Szkoła analizy jakościowej" (School of Qualitative Analysis) and "Szkoła analizy ilościowej" (School of Quantitative Analysis), were much used.

WIKTOR WAWRZYCZEK

NEWS and VIEWS

National Institute of Medical Research: Prof. P. B. Medawar, C.B.E., F.R.S.

PROF. P. B. MEDAWAR, professor of zoology at University College, London, who is to succeed Sir Charles Harington in 1962 as director of the National Institute of Medical Research at Mill Hill, brings to the post an outstanding record in medical research and an exceptionally broad background of relevant biology. A product of the Oxford Department of Zoology and Comparative Anatomy, he began research in Sir Howard Florey's department with a study of growth and movement in cell populations. When the Second World War came, he turned to nerve regeneration with Prof. J. Z. Young's group, and the problems of nerve grafting soon led him to skin grafting. Ever since, though there have been many interesting by-products of his active mind concerning human growth, wound-healing, ageing and cell transformation, he has pursued the problems of the homograft reaction, tenaciously and resourcefully. He has immensely clarified this field, and has been the leading figure in an international movement which is contributing fundamentally to knowledge of immune mechanisms. His success in research has been due to an unusual range of gifts: skill and speed in the laboratory, a flair for the design of crucial experiments, and a powerfully analytical mind. He was elected to the Royal Society at an unusually early age in 1949, and received a Royal Medal in 1959. His work is communicated in an irresistibly brilliant style, and his powers of exposition have often been used effectively to a wider public, most recently in the Reith Lectures. Somehow he has combined his activity in creative science with a great amount of work in scientific policy-making, notably on the University Grants Committee (1955–60) and the Agricultural Research Council (since 1952). His ability to combine productive research with heavy administration assures the success of his new appointment.

The London Research Station of the Gas Council: Dr. R. H. Griffith

Dr. R. H. Griffith, director of research at the London Research Station of the Gas Council, has retired. Dr. Griffith went to Magdalen College, Oxford, and obtained a D.Phil. in 1925. In the same year he joined the research laboratory of the Gas Light and Coke Company. During the ensuing thirty-five years, Dr. Griffith has built up a world-wide reputation in the intricate field of catalysis, and has contributed a number of important papers on various aspects of this subject, notably in the *Proceedings* of the Royal Society. He is the author of a standard text-book on "Contact Catalysis" and also of "The Practice of Research", which describes in detail the functions, structure and organization of a chemical research department attached to industry. In 1945 he was appointed senior research chemist to the Gas Light and Coke Company, a position he continued to hold with the North Thames Gas Board after nationalization. On the retirement of Dr. Hollings in 1952, Dr. Griffith was appointed controller of research of the Board and director of the London Research Station of the Gas Council. Dr. Griffith has been awarded the H. E. Jones Medal of the Institution of Gas Engineers on two occasions. The Gas Council is now to review its research activities, and for the time being Mr. G. U. Hopton, senior research chemist, will be responsible for research at the Fulham Laboratories of the London Research Station.

Hector: a New Research Reactor

The United Kingdom Atomic Energy Authority has now issued the contracts for the construction of a new zero-energy oscillator reactor which is to be built at the Atomic Energy Establishment, Winfrith. The reactor, which will be called *Hector* (heated experimental carbon (graphite) thermal oscillator reactor), has been designed expressly to facilitate the use of the pile oscillator technique for evaluating the nuclear behaviour of fuels, moderators, and structural materials in reactors. This technique, which utilizes small samples, has been devised by the Authority to further the reactor physics aspects of power reactor development. It is expected that *Hector* will go critical during 1962. The reactor possesses a unique feature, in that it is possible to reproduce closely the conditions appropriate to many different designs of power reactors. The reactor core is an annulus of graphite and highly enriched uranium/aluminium alloy within which various assemblies of fuel and moderator may be constructed around a central tube. The sample under investigation will be cycled through this tube, producing oscillations in reactor power-level from which the nuclear behaviour of the sample, under the conditions reproduced by the assembly, can be determined. Since the fuel material in the assembly may be natural or enriched uranium-235, plutonium, thorium, uranium-233 or irradiated elements from power stations, and since the assembly can be heated to 450° C., it is possible to investigate samples under the conditions to be found throughout the life of many different designs of power reactors. Successful measurements are possible only if the temperature of the heated assembly is extremely stable. It is desirable that the moderator temperature should not change by more than 3×10^{-3} degree C. per minute. The design of the reactor to achieve this has involved the use of novel techniques, specialized materials and the highest standard of control and instrumentation.

Special Assistance for Effluent Liaison

A three-year project to assist tanners and dressers with effluent problems has just been started by the British Leather Manufacturers' Research Association. The project has been made possible by the agreement of the leather industry, through the United Tanners' Federation and the British Leather Federation, to contribute 'new' industrial income for 'effluent liaison'. To this the Department of Scientific and Industrial Research will add £2 for every £1 of 'new' income up to a maximum of two-thirds of the total cost (estimated to be about £20,000 for the three-year period). The terms on which the grant has been awarded are that the industrial income must be additional to any other income which the British Leather Manufacturers' Research Association receives, and that it must be used for liaison purposes only. It was decided that under this scheme, two new liaison officers, one a specialist in vegetable tanning, the other a specialist in chrome tanning, should be appointed to recommend and demonstrate to supporting firms modifications in leather-making processes designed to reduce effluent problems. A mobile laboratory will be equipped to undertake the analytical tests required in this work.

British National Lending Library for Science and Technology

Changes in the book-buying policy of the National Central Library recently adopted by the Trustees, partly in consequence of the creation of a new National Lending Library for Science and Technology, are described by Mr. S. P. L. Filon, librarian and secretary to the Trustees, in an article contributed to the *Library Association Record* for August. More attention will be devoted to earlier British books (before 1958), particularly those published since 1800, when they are actually required and not available. Many foreign books not available in Britain will in future be bought, rather than depend on foreign loans. Books in English, published in other countries and in the Commonwealth, will remain a substantial proportion of those purchased by the Library, and generally the same conditions as for British books will be applied. Consideration of the purchase of medical books has been deferred until the policy of the National Lending Library for Science and Technology in this respect is clarified, and acquisition of research material, both books and periodicals, in the field of science and technology has been mostly left to the future National Lending Library. The Library will acquire foreign university theses if these are not available in Britain. In future, the Library will acquire all current British Government publications as well as useful older publications and certain publications of international bodies obtainable through H.M. Stationery Office. In future, priority will be given to the acquisition of publications of learned societies, both British and foreign. As regards periodicals, it is expected that virtually every periodical in science and technology will be taken by the National Lending Library for Science and Technology, or by some library in association with it. The position in the humanities will be very different, and while the Trustees envisage that the National Committee on Library Co-operation may wish to organize a scheme for coverage of British periodicals, they have authorized the librarian to acquire foreign

periodicals where these are not available and where they are not more suitably taken and made available by another library. The policy regarding acquisition of periodicals for loan will be closely related to that for the purchase of learned society publications. It is also intended to build up a collection of specimen copies of new periodicals. Mr. Filon stresses that these changes imply no changes in the rules for the inter-lending work of the Library or in its work for adult classes.

Index Chemicus

A NEW journal entitled *Index Chemicus* has been published by the Institute for Scientific Information, 1122 Spring Garden Street, Philadelphia (annual subscription rates: educational foundations, 250 dollars; industrial and Government agencies, etc., 500 dollars). The publication is a monthly index of new compounds reported in the literature and claims to give information within thirty days of its first appearance. The first number reports more than 3,700 newly synthesized organic compounds. The production is in the hands of a staff of experienced organic chemists, and the preparation uses both manual and machine methods for handling chemical information. A system of checking to ensure accuracy is in use. The text lists chemical names, structural diagrams and molecular formulæ, as well as complete bibliographical information including article title, authors' institutions, addresses, and original journal references for each compound. Indexes will be issued quarterly and annually. Each entry is numbered and there is an inserted author index referring to these numbers. It is noticed that Japanese and Russian literature is extensively covered.

There is no doubt that this new publication will greatly facilitate the work of research chemists in the field of organic chemistry. In other abstract journals there is a serious time lag between the publication of the material and the appearance of the abstract, and particularly in the publication of indexes. The amount of information which can be extracted from the *Index* is, however, very limited, but enough is probably given to enable a chemist to know if the publication listed is likely to be of interest to him. Particulars of prices, etc., also a booklet and specimen pages, can be obtained from the Institute.

Nuclear Engineering Abstracts

THE appearance of yet another abstracting journal is an event which it is natural to regard with some suspicion. However, the practice of nuclear engineering involves the drawing together of so many different sciences that there has been a growing need for a journal which attempts to cover the whole field. *Nuclear Engineering Abstracts* sets out to provide informative abstracts of all the important publications which are relevant to the theory or practice of nuclear engineering; the coverage is wider than that of many abstracting journals, since newspapers and house journals are included, when appropriate, in addition to the usual periodicals, patents and reports. The standard set by the first issue of the new journal is high; the layout is good, so also are the indexing and cross-referencing. If this standard can be maintained in succeeding issues, *Nuclear Engineering Abstracts* will be essential reading for anyone taking a real interest in the field (*Nuclear Engineering Abstracts*, 1, No. 1; July 1960. Pp. 100. London: Silver End Documentary Publications, Ltd., 1960. Annual subscription covering four issues, post paid, £6 6*s*.; 18.50 dollars. Single copies, if available, post paid, £1 12*s*. 6*d*.; 4.75 dollars).

Scientific Papers of the Meteorological Office

"Airborne Measurements of Latitudinal Variation of Frost-point Temperature and Wind" is the subject of the first number of a new series entitled "Scientific Papers" to be published by the Meteorological Office (Air Ministry: Meteorological Office. Scientific Paper No. 1. Pp. ii+30. (M.O. 664.) (London: H.M. Stationery Office, 1960.) 3*s*. 6*d*. net). This series replaces the former "Professional Notes" and "Meteorological Reports", and like them each number will contain one paper. It will give accounts of research work carried out in the Office. It is printed on paper more than an inch wider than its predecessors in order to allow more space for the diagrams which are an important feature of most meteorological papers. Accounts of the most important investigations will, however, continue to appear in the "Geophysical Memoirs" series. This first issue, by N. C. Helliwell, summarizes and discusses observations of the elements quoted made by the Meteorological Research Flight in the lower stratosphere on six days in the summer of 1956 and three in March 1957 between Minorca and the Norwegian Sea near the Arctic Circle. The frost-point, giving a measure of the humidity, showed little variation with latitude. Jet streams were traversed on some flights, and the horizontal wind-shear in them was measured. Clear-air turbulence was, as expected, encountered in association with some jet streams, but it was also encountered at places where no explanation for it could be found. The temperature observations on two flights were such as to suggest that tropospheric fronts were continued into the stratosphere.

Environment and Culture in Southern Rhodesia

ROGER SUMMERS, curator of the Museum at Bulawayo, Southern Rhodesia, has published an interesting paper on the relationships of environment to past cultures in that country (*Proc. Amer. Phil. Soc.*, 104, No. 3; June 15, 1960). In arguing from the present to the past, changes in climate naturally have to be remembered, but the physical map and the geology of the region have remained constant. No less than twenty-three distribution maps are given, showing such things as the modern vegetation pattern and what it would be with various percentages of the present rainfall. Other maps show the distribution of the prehistoric cultures, as well as of the rock-shelter art and the ancient mine workings. Why did some early cultures shun areas where Mopane forests would be growing? The answer is probably because the tsetse fly *Glossina morsitans* would have made cattle breeding impossible in those districts. The various possible invasion routes depend to a large extent on the 'lay-out' of the country, and peoples coming in from outside have, of course, greatly influenced past history. Actually, Southern Rhodesia can be shown to have been both a cradle and a corridor. Again and again not enough attention has been paid to the relationship of various culture groups to their environment, both climatic and geological, when the past history of a country is being studied. Roger Summers is to be congratulated in having so successfully followed in the footsteps of Sir Cyril Fox in producing for Southern Rhodesia what Sir Cyril did in his "Personality of Britain".

Strontium-90 and Natural Strontium in Human Bone

A recent report, "Radioactive and Natural Strontium in Human Bone", from the Chemistry Division of the Atomic Energy Research Establishment, Harwell, is the latest in the series in which results of the analyses of human bones in the United Kingdom for strontium-90 and natural strontium are listed (A.E.R.E. *R*23246. By J. W. Arden, F. J. Bryant, E. H. Henderson, G. D. Lloyd and A. G. Morton. Pp. 8. London: H.M. Stationery Office, 1960. 1*s*. 9*d*. net). The results given are for samples received during 1959 and analysed by the end of the year. Many of the samples were from subjects who died during 1957 and 1958. For the determination of strontium-90 the separation and counting of the yttrium-90 daughter is the preferred method, but when the activity is too low the determination has to depend on counting of the strontium source. Emission spectroscopy was used for the determination of the natural strontium sources. The results show that between the latter part of 1958 and the first half of 1959 there was a rise, compared with previous periods, in the number of strontium units absorbed for all the various age groups except the 'over 20 years' group. The contamination of diet in the United Kingdom is largely dependent on the rate of fall-out and the higher bone burdens thus reflect the increased rate of deposition which has occurred since early 1958.

Low-temperature Fuel Cell

The successful development of a low-temperature fuel cell that can generate 3–5 times more power per unit volume than other similar cells has been announced by the Shell Research Centre at Thornton, Cheshire. The high output has been achieved as a result of fundamental studies on fuel cell electrodes. This work has led to the development of inexpensive, highly active electrodes. The cell can operate with either alkaline or acid electrolytes. When run on hydrogen and oxygen at a pressure of 3 lb. per sq. in., current densities of 70 amp. per sq. ft. can readily be obtained with both types of electrolyte at room temperature. If air is substituted for oxygen there is little loss in performance. As the cell warms up to 60° C., the output is doubled and a power output of 3–5 kW. per cu. ft. is achieved. Multiple fuel cells with up to six cells per inch have been constructed, and it is considered that units with a power-to-weight ratio of 50 W. per pound could be made. The work at Thornton is continuing and will include a study of fuels other than hydrogen. During the past decade, much of the effort on fuel cells has been devoted to the development of high-temperature cells since, in these cells, the electrode reactions are rapid and high efficiencies can be more easily achieved. Recently, however, more attention has been given to low-temperature cells and it would appear that this is a fruitful field for further research and development.

Travelling Fellowships in Medicine

Applications are invited for Rockefeller travelling fellowships, Sir Henry Wellcome travelling fellowships, U.S. Public Health fellowships and the French exchange scholarships. These awards are open to graduates in science who are resident in the United Kingdom and have some training in research in medical science and who intend to return to Britain to resume or take up new appointments in university, hospital or academic research. Fellowships held in the United States provide annual maintenance allowance at rates varying from 3,000 dollars according to individual circumstances (500–700 new francs monthly with additional sterling marriage allowance in the case of French exchange scholarships) and travelling expenses are also provided. Further information and application forms may be obtained from the Secretary, Medical Research Council, 38 Old Queen Street, London, S.W.1, and the closing date for applications is October 31.

Announcements

Mr. A. R. O. Williams, a managing director of Consolidated Gold Fields, Ltd., and of New Consolidated Gold Fields, Ltd., has been elected president of the Institution of Mining and Metallurgy for 1961–62. He has served on the council of the Institute for fifteen years, and held the office of vice-president during the period 1951–54.

Dr. W. J. Kroll, of Corvallis, Oregon, will deliver the sixth Castner Memorial Lecture, "The Fusion Electrolysis of Titanium", on October 7 at the Royal College of Science and Technology, Glasgow, C.1. Further information can be obtained from the Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

The Research Laboratories of the Timber Development Association at St. John's Road, Tylers Green, Penn, Buckinghamshire, are holding two open days on October 12 and 13. Further information can be obtained from the Association's headquarters, 21 College Hill, London, E.C.4.

There will be open days at the National Chemical Laboratory, Teddington, Middlesex, on October 19 and 20. Applications for invitations should be sent to the Director not later than September 24. Those already on the mailing list for invitations need not re-apply.

The next meeting of the Biochemical Society will be held in the Department of Biochemistry, the University of Glasgow, during September 29–30, and will include a colloquium entitled "The Biosynthesis of Nucleic Acids". Further information can be obtained from Dr. F. A. Robinson, c/o Allen and Hanburys Ltd., Bethnal Green, London, E.2.

Joint Conferences are to be held on "Analytical Chemistry in Nuclear Reactor Technology" and "Nuclear Reactor Chemistry" at the Civic Auditorium, Gatlinburg, Tennessee, during October 12–14. The Conferences are sponsored by the Analytical and Reactor Chemistry Divisions of the Oak Ridge National Laboratory. Further information can be obtained from C. D. Susano, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tennessee.

The Aero Space Medical Panel of the NATO Advisory Group for Aeronautical Research and Development (AGARD) will hold its sixteenth meeting in Istanbul, Turkey, during October 3–8. Technical papers on clinical aviation and space medicine will be presented. Further information can be obtained from the Advisory Group for Aeronautical Research and Development, Organisation du Traité de l'Atlantique Nord, 64 rue de Varenne, Paris 7ème.

Erratum. In the article entitled "Blue Hazes in the Atmosphere" by Dr. F. W. Went, in *Nature* of August 20, p. 641, the sizes of particles mentioned are incorrectly given in 'mμ'; they should be 'microns'.

WEATHER PROBLEMS IN AGRICULTURE

FOUR papers were presented during the second morning (July 1) of the summer meeting of the Royal Meteorological Society at Queen's College, Dundee.

Introducing the session before presenting the first paper, Dr. H. L. Penman, of Rothamsted Experimental Station, stated that two of the main objectives of crop-weather studies are, first, to achieve better growth of healthy plants, and secondly, to exercise control of pests and diseases the activity of which is dependent on weather. It is essential for the agricultural meteorologist to know something of the principles of plant-growth, though two of his problems can be derived from the simple equation for photosynthesis of carbohydrate. The need for short-wave radiation made a study of solar radiation income imperative, both as physics and as climatology. The latter topic was taken up later by Mr. G. J. Day. The supply of carbon dioxide to the crop can now be treated as a meteorological phenomenon similar to heat and vapour transfer (see second paper, by Dr. J. L. Monteith), and out of it there arises a third problem. The physiological state of the plant that permits easiest uptake of carbon dioxide also provides the most favourable conditions for transpiration, and this transfer of water from soil to atmosphere presents the meteorologist with another flux problem, and with a problem in energy balance. To the micro-meteorologist it brings problems concerned with the source of the water, and the effects that it has on the immediate environment of the plant, that is, on the 'weather' experienced by the plant, its pests and its pathogens.

In the hope that the information would some day be biologically useful, Dr. Penman and Mr. I. F. Long have been collecting detailed physical measurements in various farm crops. "Weather in Wheat"[1] is a condensation of the results for three summers of temperature, humidity and wind-speed, all continuously recorded over and within the crop on a site on which many other auxiliary measurements were made. Selecting a few topics, primarily of meteorological interest, a demonstration of variability across the site at constant height provides one of the reasons for using long-period averages in the analysis of profiles. Hot spots and damp spots can persist for several hours. By plotting wind-speed against temperature, and against vapour pressure, at the four observation heights above the crop, straight lines were obtained, from which it was inferred that the aerodynamic transport constants were effectively equal for transfer of momentum, sensible heat and latent heat. Computation of the transfer of latent heat for the forty-eight 4-hr. periods in eight days of fine weather gave very good agreement with the measured values from a large transpiration gauge, continuously weighed, in phase, amplitude and in mean daily total. Both estimated and measured values showed that transpiration is a daylight phenomenon. Calculations were presented for two periods to show how the transpiration stream was built up, layer by layer, between the soil surface and the top of the crop. It seemed that all layers contributed about equally (in this particular crop). Some time in the future it may be possible to tackle the carbon dioxide problem in the same way and find out the vertical distribution of efficiency of assimilation.

Dr. J. L. Monteith, also from Rothamsted, then summarized what he and Mr. G. Szeicz had done in measuring "The Carbon Dioxide Flux over a Field of Sugar Beet"[2], work first made possible by a grant from the Rockefeller Foundation and later by the skill of the Infra-Red Development Co. in building an instrument capable of measuring to 0·1 p.p.m. of carbon dioxide. From differences of concentration at two heights 25 cm. apart above the crop, and contemporary wind data, fluxes were computed. For a period of a few weeks from August onward the flux was always downward in daylight (assimilation > respiration), the main changes following very closely behind those of solar radiation intensity. The night flux was upward, at a fairly constant rate representing plant respiration and biological action in the soil. Net growth over the period calculated from these physical measurements agreed well with direct measures by crop sampling. Later, in October, biological activity in the soil appeared to provide more than enough carbon dioxide for plant growth—the day-time flux was upward except during short sunny intervals around noon, and the crop was still growing. Though this latter behaviour raised some important problems in soil microbiology, Dr. Monteith confined his discussion to the meteorological aspects of the earlier period, giving a hint that he had since made progress in the interpretation of the interactions of radiation intensity, mixing parameters and carbon dioxide uptake, and that there was a possibility of a link-up between the field-work he and Szeicz were doing, and laboratory work on single leaves being done elsewhere. As a purely meteorological point, he suggested that the uniform upward flux at night might be exploited in the study of transfer processes in very stable conditions. In discussion, the somewhat optimistic suggestion was made that this physical technique might replace periodic sampling, to give a non-destructive method of measuring the rate of plant growth.

Although there are many stations with long records of duration of bright sunshine (n), it is only within the last few years that there has been any attempt to measure incoming solar radiation (Q) at stations in Britain other than in south-east England. There has been much use of an empirical equation $Q/Q_A = a + bn/N$, in which Q_A is the radiation that would reach the site in the absence of an atmosphere, and N is the maximum possible duration of sunshine. Under the title "Radiation Climatology", Mr. G. J. Day (Meteorological Office, Eskdalemuir) first gave the values of a and b he has derived from monthly mean values of Q/day and n/day obtained at twelve stations having records of Q for at least three years. The geographical distribution of stations was very irregular, with large gaps in northern Scotland, eastern Scotland, eastern England, the Welsh border and along the south coast. Mr. Day made reservations about the accuracy of some of the

radiation records, but by giving equal weight to all observations he found that a has a range from 0·08 to 0·19; and b varies from 0·63 to 0·95. Neither constant shows any systematic geographical variation. Having got local values of a and b from short-period records, he applied them to large neighbouring areas with long-term records of n, to derive expected mean monthly average values of Q. These were plotted together with the corresponding values given by Black[3] for twenty-eight stations in the area bounded by lat. 50–60° N., long. 5–30° E., and isopleths of mean monthly radiation drawn for north-west Europe using the two sets of estimates. The value of the twelve maps lies in their detail, and this may be modified after a re-check of some of the basic data. When published, they will be a very useful supplement to the corresponding maps of rainfall, temperature and sunshine.

During recent years there has grown a demand for more information about the effects of shelter on plants and on animals. Much of the resulting field work has the active co-operation of the Meteorological Office in Edinburgh, and in a paper entitled "Windbreaks—Some Physical Effects", Dr. R. W. Gloyne of that Office considered some purely aerodynamic aspects of air flow over a barrier. Summarizing world experience, he listed the three distinct flow regions that arise: (1) the up-wind boundary layer; (2) the down-wind disturbed region; and (3), overlying these, the undisturbed flow. The disturbed region can be subdivided into: (2a) a space, immediately behind the obstacle where there may be strong eddies—it is here where it is most difficult to reconcile field and wind-tunnel studies; (2b) a region, farther down-wind, of turbulent motion, approximately conforming to a logarithmic profile, which, with distance, becomes in fact the logarithmic profile of the original undisturbed flow. The sizes of the regions (as multiples of the barrier height h) vary with the permeability (φ) of the barrier. In brief (with winds of normal incidence): for dense barriers ($\varphi < 40$ per cent) the original profile is re-established at about $30h$, though a logarithmic profile may re-appear at $20h$, and the original mean direction is re-established at about $15h$. For open barriers ($\varphi > 60$ per cent) the re-establishment of speed profiles is much as for dense barriers, but that of mean direction occurs much nearer the barrier, perhaps as close as $2\frac{1}{2}h$ behind a very open mesh. The width of the affected area depends on the length (L) of the barrier. When $L > 20h$, the system behaves almost as a portion of an infinite barrier; a shorter barrier has relatively larger edge effects so that the affected area varies roughly as L^2, approximating to a parabola with its vertex perhaps $1\frac{1}{2}L$ down-wind.

Dr. Gloyne concluded with a brief reminder that there is a pressure excess up-wind and a pressure deficiency down-wind of an obstacle placed in an air stream, and he suggested that pressure measurements, similar to those made in wind tunnels, might be useful in wind-break studies. H. L. Penman

[1] Penman, H. L., and Long, I. F., *Quart. J. Roy. Met. Soc.*, **86**, 16 (1960).
[2] Monteith, J. L., and Szeicz, G., *Quart. J. Roy. Met. Soc.*, **86**, 205 (1960).
[3] Black, J. N., *Archiv. für Met., Geophys. und Biokl.*, B**10**, 2 (1960).

TUBERCULOSIS IN ANIMALS

TUBERCULOSIS in animals was the subject of the fourth symposium of the Zoological Society of London, held on May 24. Mr. J. N. Ritchie, who organized the meeting, presided at the evening session. Dr. R. E. Glover was chairman at the morning session, and in the afternoon Dr. J. M. Ross filled this position.

Dr. Glover explained that the subject was appropriate for discussion at this particular time because of the progress that had been made in the eradication of tuberculosis from the cattle in Great Britain. With this advance, it was useful to review the position among other animals.

The first paper was by Dr. J. T. Stamp, who dealt with the comparative pathology. In calves, the incidence of tuberculosis is low, and infection enters mainly by inhalation or is congenital. Hæmatogenous dissemination is frequent. The fact that the incidence of tuberculosis increases considerably with age shows conclusively that tuberculosis of the adult is not merely a slow progression of lesions acquired in calfhood. In the older animals, the site of the primary complex is predominantly pulmonary and it is very obvious that advanced pulmonary tuberculosis develops as a result of bronchial dissemination following cavitation of the primary lung focus. The pathology of tuberculosis of the udder shows that there is always extensive involvement of the milk ducts.

In the pig, the chief route of infection is by the alimentary tract, and this gives rise to an incomplete primary complex in the regional lymph glands. In bovine-type infection in this species, hæmatogenous dissemination is common, but chronic pulmonary tuberculosis occurs only infrequently.

Horses have a high resistance to tuberculosis. Primary infection is almost entirely by ingestion. Hæmatogenous dissemination is frequent and gives rise either to miliary tuberculosis or to large tumour-like swellings, not unlike those of sarcoma.

The tuberculin test and the laboratory diagnosis of tuberculosis were discussed by I. W. Lesslie, who gave a brief history of the methods of preparation of tuberculin and the ways in which it had been used.

The general objective of research on tuberculin is to obtain a preparation which will give a maximum response in the infected animal at all stages of sensitization and a minimum response in the non-infected or non-specifically sensitized animal. He described the procedure necessary for the characterization of tuberculins.

The intradermal comparative tuberculin test has been developed for use in cattle. It consists in the injection of 0·1 ml. of avian and mammalian tuberculins in selected sites in the neck. The reaction at each site is measured, and the test is interpreted on each animal with reference to the results recorded in the whole herd and having regard to the herd history and clinical findings. *P.P.D.* tuberculin is used (mammalian 2·5 mgm./ml., avian 0·5 mgm./ml.). The relative strength of the *P.P.D.* tuberculins resulted from somewhat empirical decisions based on the strength of the tuberculins formerly used in this test.

A laboratory service is of great value in confirming field diagnosis and, particularly, for typing the organisms concerned. Frequently, when re-infection in a herd of cattle is being investigated, the knowledge of the type of organism involved is helpful in tracing the source of infection, both in animals and in man.

Mr. J. D. Blaxland, in describing tuberculosis in poultry, said that it is no longer of great importance as a cause of serious loss in our flocks. This relates generally to the lower age of birds being kept and the better conditions of husbandry. Nevertheless, tuberculosis still accounts for a fairly high proportion of condemnations of poultry carcases. The disease affects domestic and wild birds. There is no good evidence that wild birds play any large part in infecting domestic species, but apparently wild birds do acquire infection from poultry. Wild birds may, however, be responsible for infecting farm animals.

Organisms of the avian type may cause progressive disease in pigs, and account for about one-third of the reports of tuberculosis in British pigs. Avian-type infection in cattle is important because it sensitizes the animal to tuberculin and, unless some form of comparative tuberculin test is used, may lead to errors in diagnosis.

Mr. I. M. Lauder, discussing tuberculosis in dogs and cats, mentioned that the incidence is much reduced. Bovine-type infection is now rare among cats, but a few cases of human-type infection occur. Tuberculosis was found in 1–2 per cent of autopsies of dogs in veterinary schools in Great Britain in the last five years. Infection in dogs is mainly of human-type and a source in man has been found in 77 per cent of cases, usually within the household. Symptoms in dogs are readily recognizable, and in these animals diagnosis may be made by X-ray examination. The tuberculin test is not reliable but a test using B.C.G. vaccine intradermally is of some value. Many of the comparatively few cases of tuberculosis found in dogs are 'open' and humans might therefore be infected from this source.

In a paper on the disease in zoo animals, Mr. Oliver Graham-Jones emphasized that the primates are very susceptible, and it is common in all vertebrate species. All three main types of the organism are involved. Many carnivores, which might be expected to acquire bovine tuberculosis from eating infected meat, instead are found to be infected with a human strain apparently from close contact with the general public.

Bovine infection has been recorded in monkeys, although one would expect the infection to be with the human strain entirely.

There is considerable difficulty in diagnosis because the usual signs of loss of condition leading to emaciation are not so apparent in animals in captivity. The use of laboratory techniques in diagnosis is becoming more important; but it is recognized that a suitable diagnostic test is needed. The only test available at present is the intradermal tuberculin test, as developed for cattle. There is some promise, however, from the use of B.C.G. vaccine which was developed by the author as a diagnostic aid in primates and has since been used in dogs.

Treatment is, of course, possible; but it was stressed that eradication of the disease by destruction of the affected animal, and prevention of recurrence by strict measures of disinfection was the best practical course. Precautions have, of course, to be taken by testing incoming animals with tuberculin. So far as possible, this should be done before the animals leave their country of origin; but this is sometimes quite impracticable. Whatever the practical difficulties of carrying out tuberculin tests and eradicating the disease from zoo animals, it was most important to appreciate that in the light of the current successful eradication programme for tuberculosis in cattle, it is most undesirable that zoological gardens should remain a reservoir of infection which might overspill into rural areas.

Mr. R. V. Blamire, dealing with tuberculosis and food inspection, pointed out that although tuberculosis has, in the past, been regarded by food inspectors as the most important animal disease, its significance has rapidly declined in recent years in Great Britain. More than 97 per cent of milk consumed is now either heat-treated or comes from attested herds.

The techniques of meat inspection are exacting, and the judgment of tuberculous carcases is often difficult.

In 1957, approximately 7·3 per cent cattle, 0·1 per cent calves, 3·6 per cent pigs, and 0·0006 per cent sheep were found to be affected with tuberculosis when inspected in abattoirs in England and Wales. In 1950, it is estimated that the value of meat rejected because of tuberculosis was about £1,000,000; in 1955, although the price of meat had doubled, £800,000, and in 1957 it was £500,000. Soon, the loss should be negligible.

The risk to public health from the consumption of under-cooked tuberculous meat has never been clearly established; but it should not be under-estimated. The detection of tuberculosis by meat inspectors will in future have more animal health than public health significance. Differential diagnosis will then become very important.

The disease in man was dealt with by Prof. J. W. Crofton.

With the greater control of malaria, tuberculosis has become the world's most important public health problem. The World Health Organization calculates that 0·5–1 per cent of the entire population of the world is coughing up tubercle bacilli at any one time. Since the introduction of modern chemotherapy in 1947, there has been a dramatic fall in mortality from tuberculosis, at least in the economically developed countries; but the fall in notifications has been very much slower. Human infection by bovine tubercle bacilli can be measured directly only by typing bacilli isolated from patients. Indirect estimates can be made by following trends of tuberculosis in young children, who are likely to be infected from milk, or of such forms as abdominal tuberculosis which are likely also to be associated with milk infection. Figures quoted demonstrated such falls, greater in urban areas, where milk infection was first well controlled, than in rural areas, and much greater than the falls in respiratory tuberculosis.

The control of infection with human bacilli depends on efficient case-finding, mainly by mass miniature radiography, on efficient treatment to render patients permanently non-infectious, on efficient prophylactic vaccination with B.C.G. and on the general improvement in environmental conditions. Figures for Edinburgh were quoted to show the dramatic effect relatively thorough re-organization and good teamwork can have not only on the mortality but also on the morbidity of tuberculosis. It is suggested that, with sufficient energy and enthusiasm, the elimination of tuberculosis could be proceeding much more rapidly than it is in most areas at the present time.

Mr. W. D. Macrae traced the story of the eradication of tuberculosis from cattle from its beginning with the introduction of the Tuberculosis Attested Herds Schemes in 1935. It proved relatively easy to reduce an incidence of 20 per cent to 0·3 per cent; but there is real difficulty in reducing this figure any further for various reasons, such as re-infection from other animal sources, including man; loss of tuberculin reactivity at certain stages of the disease; and residual infection on farms, particularly in old, dilapidated buildings.

The overall current incidence of infection is 0·16 per cent of the animals tested but in some areas it is now as low as 0·05 per cent. Whether it will ever be reduced beyond this figure is a matter for conjecture.

Among the reactors disclosed, some 70 per cent show evidence of disease at postmortem examination in abattoirs. This figure of confirmation was higher at one stage but has fallen somewhat as a result of difficulties in carrying out adequate postmortem examinations and the apparent increase in the number of reactors showing no visible lesions.

In the course of dealing with bovine tuberculosis, the incidence in pigs of the bovine type infection has been markedly reduced and may well disappear in this species.

A great deal of effort is still required to maintain the disease under strict control. There must be regular organized tuberculin testing at appropriate intervals and tracing of animals which have been in contact with infection, so that the herds to which they have moved may be tested. Every source of information of the presence of tuberculosis in cattle must be used, including abattoirs and knackeries.

Tuberculosis in cattle, although not eradicated in a literal sense, is at least under satisfactory control, and there is every promise that it will be maintained at an extremely low incidence in future. Coincidentally, the amount of infection among other animals has been reduced in parallel, but certain precautions are still necessary to prevent re-infection of herds of cattle. Man may remain a source of infection, and while bovine-type tuberculosis in man is probably not sufficiently common among people who are in close contact with animals to make this a serious hazard, it has to be appreciated that as the interval between tests of herds is extended any source of re-infection is very important.

THE BRITISH GELATINE AND GLUE RESEARCH ASSOCIATION

THE nineteenth meeting of the Research Panel of the British Gelatine and Glue Research Association was held on May 19, with the chairman of the Association, Mr. S. G. Hudson (Richard Hodgson and Sons, Ltd.), in the chair.

Dr. G. R. Tristram (Department of Physiology and Biochemistry, University of St. Andrews) presented "A Preliminary Study of the Thermal Conversion of Acid-soluble Calf-skin Collagen into Gelatin". The collagen was obtained by extraction of calf-skin with cold aqueous acetic acid. The release of end-groups on heating with water at *p*H 6 was investigated, using Sanger's method. Changes in optical rotation and viscosity caused by heating at *p*H 3·3 in 0·1 *M* aqueous acetic acid solution were recorded. At *p*H 6, most of the collagen dissolved after 1 hr. at 50° C. and all of it after 1 hr. at 60° C. Solubilization was accompanied by a large increase in ε-amino groups reactive towards Sanger's reagent and by the appearance of terminal amino-groups of a similar pattern to those found in eucollagen by Courts[1]. End-groups were also estimated after keeping the collagen in strong aqueous urea at 15° C. so as to obtain a comparison with the effect of heating in water. On heating at *p*H 3·3 the optical rotation and viscosity rose to a maximum at about 28° C. and then fell. Dr. Tristram drew some tentative conclusions about the unmasking of N-terminal residues during the treatments applied and discussed the question of there being one or more chains in the collagen structure.

The second paper, "The Swelling of Gelatine Films", by Miss E. M. Simms and Mr. J. N. Blake (Richard Hodgson and Sons, Ltd.), was presented by Mr. Blake. A variety of gelatine gels and sols were dried on a solid supporting base at various temperatures in the range 5–60° C. The resultant thin films were detached and allowed to swell freely in water at 10° C.; previous workers in this field have studied attached films. The weights and dimensions of the swollen films were measured. By far the most important factor controlling swelling was the temperature at which the films were dried; for example, a 210*g*. Bloom acid-process pigskin gelatine film, imbibed twenty-three times its weight of water if dried at 5–10° C. but only six times if dried at 30–35° C. The imbibition was largely evidenced by an increase in thickness rather than in linear dimensions, whereas films dried from the sol state exhibited the opposite behaviour. Swelling in water at 20° C. was considerably greater than at 10° C. and swelling was more pronounced in films dried from weak (5 per cent) solution than from strong (20 per cent) solution. Films dried unrestrained on liquid surfaces from both sols and gels exhibited subsequent swelling of the sol form type. The extensive results were discussed in terms of the molecular orientation of the systems during drying.

Mr. A. M. Kragh (British Gelatine and Glue Research Association) gave the final paper, entitled "The Flocculation of Suspensions with Gelatine". Gelatines and animal glues have a flocculating action on a wide variety of suspended solids of which quartz has been most studied, and this property has been evaluated in comparison with that of other flocculating agents both polyionic (for example, synthetic polyamides) and non-ionic (for example, dextran). Gelatine flocculates best where almost all of it is adsorbed on to the particle surfaces and where the amount so adsorbed is about one-third of the maximum adsorbable. It is exceptional among flocculating agents in its ability to be strongly adsorbed on a wide range of different surfaces and in that its flocculating action is strongly dependent on *p*H. The lively discussion centred around the mechanism by which flocculation is induced in these systems.

[1] Courts, A., *Biochem. J.*, **74**, 238 (1960).

DOMESTIC HEATING AND THERMAL INSULATION

DIGEST 133 of the Building Research Station, entitled "Domestic Heating and Thermal Insulation" (Pp. 7. London : H.M. Stationery Office, 1960. 4*d*.), brings together the essentials needed to guide the choice of the standard and methods of heating from among the many systems that are available. In choosing a domestic heating system the decisive questions are : What standard of heating is required ? How much will it cost to run ? Convenience in operation and the form of heat distribution preferred are also important.

Broadly, the systems of domestic heating can be divided into three main categories : (1) Full house warming. (2) Partial heating, that is, the full heating of two or three rooms from a central heat-source, with the remainder of the house heated as required by gas or electric fires : or the background warming of the whole house to a uniform temperature lower than that obtained by category (1) together with 'topping up' in individual rooms as required. (3) Heating limited to individual appliances in one or two of the main living rooms.

The main differences in the three standards of heating are in the scope of the heating service. In the full house-warming systems and background heating systems, for example, comfort is enhanced by the fact that the house is uniformly warm ; such systems are essential for the open-house plan. By contrast, systems using separate appliances, for example, open fires, in a limited number of rooms give a less-uniform standard of comfort.

The standards covered by these three categories can be characterized broadly in terms of difference between the average outdoor temperature over the whole heating season and the average indoor temperatures they provide.

Assuming an average seasonal outdoor temperature of 43° F., a temperature difference of 18° F., averaged over 24 hr., can be taken as representative of full house warming, 13° F. for partial central heating systems and 11° F. or less for the third category. The smaller temperature difference between the medium and low standards of heating may be explained by the fact that even in dwellings where the bedrooms are unheated some stray heat is received from the heated part of the house. These average temperature differences can help in estimating seasonal fuel requirements and, therefore, running costs. They are not, however, appropriate for estimating maximum heat demands and hence the size of the heating plant ; these have to be calculated from a maximum temperature difference between indoors and outdoors.

For a fair comparison of capital costs of various domestic heating systems, account should be taken of any building costs incidental to the heating installation, such as those of brick flues and fuel stores, or, conversely, the saving effected if these items are not required. For example, in a system comprising one open fire with back boiler, electric immersion heater for summer use and two electric fires, the cost of a chimney breast and flue and a fuel store for the open fire is about 25 per cent of the total cost of the installations. Broadly, the capital costs of the three categories of heating are in the same order as the standard of heating attained, but there may be some overlapping.

The seasonal heat loss for a particular dwelling will depend to some extent on the overall floor area, on the grade of insulation, the standard of heating, the ventilation-rate and the length of the heating season.

To estimate the seasonal fuel requirements the seasonal loss of heat should be divided by the calorific value of the fuel multiplied by the 'house efficiency' of the heating system.

Other points affecting the choice of a heating system are convenience in operation and the form of heat distribution. The size and number of heating appliances required for a particular heat service depend mainly on the maximum heat demand during a cold spell.

POPULATION TRENDS AMONG HAWKS AND OWLS IN ILLINOIS

DATA on hawks and owls in Illinois, as reported in the Christmas counts sponsored by the National Audubon Society and its parent organizations, have been analysed by Richard R. Graber and Jack S. Golden to determine population trends for the years 1903–55 (Biological Notes, No. 41. Dept. Registration and Education, State of Illinois).

Even though most of northern and central Illinois offered a fairly uniform habitat of cultivated farm land with relatively little forest, the distribution of raptors was not uniform, and raptor populations varied considerably even within relatively small areas. Highest concentration of raptors was in the southern part of the State. Through the years a given locality, in relation to other localities, has tended to show a consistent rank with regard to the density of its raptor populations.

Analysis of frequency of occurrence for all species of raptors in the period 1903–55 was based on 527 censuses—103 in southern, 123 in central, and 301 in northern Illinois. This analysis shows the expected higher frequency of prairie-inhabiting species of raptors in central and northern Illinois and of woodland species in southern Illinois. Regardless of habitat, certain species, such as the sparrow hawk and the marsh hawk, were seen with increasing frequency from north to south, as though responsive to a temperature cline.

The combined data for all raptor species show that there has been a marked and consistent decline in winter raptor populations during the period 1903–55. Peaks representing years of high populations are lower in later years. Trends in raptor populations were deduced from birds per observer-hour, birds per party-hour, and birds per mile.

The records of individual species show that the rough-legged hawk has suffered the severest decline in numbers ; the red-tailed hawk, the sparrow hawk

and the barred owl also decreased. Marsh hawk and the red-shouldered hawk populations have not changed appreciably. The bald eagle has been increasing in numbers since the early 1940's; but this is the only species for which a notable increase was shown. The records for many species are too incomplete to evaluate.

Because the decline in raptor populations is shown particularly among the less-wary species and those species that are most likely to be encountered by man, it is suggested that man may be directly responsible for the decline. This view is corroborated by the fact that many raptor band returns are from birds that were trapped or shot and also by the indication that a notably lethargic species, the rough-legged hawk, has suffered the severest decline.

The species of raptors, namely, *Buteo* spp., *Circus* spp. and *Falco sparverius*, which have been protected by law since 1919, were seen at least seven times as frequently as the previously unprotected accipiters and the great horned owl.

SINGAPORE BOTANIC GARDENS

A SPECIAL issue of the *Gardens' Bulletin* (17, Pt. 2; 1959. 12.50 dollars. Obtainable from the Botanic Gardens, Singapore) has been issued to commemorate the hundredth anniversary of the founding of these well-known gardens. In fact, a botanic garden was established in Singapore soon after its founding in 1819 by Sir Stamford Raffles, nutmegs, cloves and cocoa being among the early introductions. This garden was closed after some years in a retrenchment of expenditure, but new gardens, the present ones, were established on a new 60-acre site towards the end of 1859 (see also *Nature*, 184, 1602; 1959).

Since then the gardens have been extended and amplified and for many years have been recognized as being among the most important tropical institutions of this kind, the more so as they are set in a region of very considerable agricultural importance and quite exceptional floristic interest. Virtually from the outset, the staff have paid attention to the needs of practical agriculture and horticulture as well as to systematic and other aspects of scientific botany usually associated with botanic gardens. This was exemplified in a remarkable degree by the work of Dr. H. N. Ridley, director of the Gardens during 1888–1912, who has not only been rightly named "the father of Malaya's rubber industry" but was also responsible, as an indefatigable field botanist, for vast accessions of new plants to the gardens and its herbarium. After his retirement, he produced his five-volume work on the "Flora of the Malay Peninsula" (1922–25).

As several distinguished botanists have worked on the staff of the Singapore Botanic Gardens over the years, the output of work, often on plants of unusual scientific interest, has been both extensive and impressive. In the present commemorative volume (pp. 125–340) the reader will find much that is pertinent, informative and interesting. J. W. Purseglove has contributed a paper on the history, functions and contemporary work of the gardens; T. A. Russell has described the relationships between them and the Royal Botanic Gardens, Kew; while H. J. Lam has written of Singapore and Leyden. Other authors deal with the contribution which work in the gardens has made to agriculture in the Malaysian region, and there are several papers of both general and special interest by well-known botanists; for example, R. E. Holttum has written on his work on orchids, gingers and bamboos carried out in the gardens, and E. J. H. Corner has discussed the importance of the taxonomy of tropical plants in contemporary botany; and so on.

Perhaps the best way of indicating briefly the value of the present volume is simply to say that, for the general botanical reader, it affords an unusually interesting introduction to the many facets and fascinations of tropical botany.

ROAD ACCIDENTS DURING CHRISTMAS 1959

TWO factors stand out in the Road Research Laboratory report of the investigation of the 150 fatal road accidents in England and Wales during the Christmas period (December 24–27, 1959)—the bad weather conditions and the unusually high proportion of people involved in the accidents who had taken alcohol (Road Research Technical Paper. No. 49. Pp. iv+20. Published for the Department of Scientific and Industrial Research by H.M. Stationery Office, 1960. 1*s*. 9*d*. net).

The investigation was carried out at the request of the Minister of Transport and shows that the number of fatalities per mile travelled by motor-vehicles was high on each of the four days of the Christmas period when compared with the other days in December or with the year as a whole.

The factors usually associated with road accidents (such as vehicle defects, cross-roads, and skidding on wet roads) were present in the normal proportion.

The weather conditions were bad. Roads were dry for only 10 per cent of the accidents; in 73 per cent it was dark and wet; in 12 per cent it was daylight and wet; and in 5 per cent it was icy. The report states: "this weather would be expected to result in increased accident frequency but not to the extent that occurred".

In at least 56 per cent of the accidents one or more of the people involved had taken alcohol not long before the accident. The corresponding figure in a normal period is 18 per cent. The report concludes: "the bad weather of the Christmas period made travelling conditions difficult. The fact that many of the people involved in the accidents had been drinking beverages containing alcohol would be expected—in the light of previous research—to have made many of them less able than usual to deal adequately with the difficult conditions. Although other factors may have been involved, the combination of these two alone might account for the high accident-rate over the period".

UPPER-ATMOSPHERE WIND STUDIES BY *SKYLARK* ROCKET SODIUM EXPERIMENT

By Dr. G. V. GROVES
Physics Department, University College, London

IN the sodium experiment, sodium vapour is ejected from a high-altitude rocket along its flight path. If the experiment is performed at twilight, when the sky has darkened yet the trail itself is still in sunlight, the trail glows conspicuously yellow by re-radiating the *D*-line emission from the Sun. This experiment, originally proposed by Prof. D. R. Bates, was first performed in 1955 at White Sands, and is now being carried out with the *Skylark* rocket at the Woomera Rocket Range.

The first *Skylark* experiment was carried out on December 3, 1958, the rocket being fired within 2 min. of a specified time, when the Sun was at a depression of 6° below the horizon. The sodium trail was laid continuously from 67 km. altitude over the trajectory vertex at 126 km., and down to a height of 93 km. Photocells on the rocket recorded sunlight from 60 km. altitude on the upward path to 69 km. on the downward path.

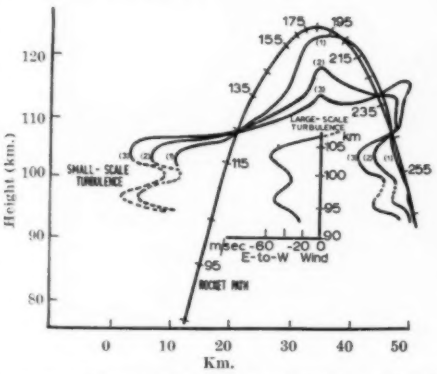

Fig. 1. East-to-west displacement of sodium trail. *Skylark* 11, Woomera, South Australia, December 3, 1958, 19.43 hr. Numbers on rocket path give time in sec. after launching. Sodium trail at (1) + 270 sec., (2) + 330 sec., (3) + 390 sec.

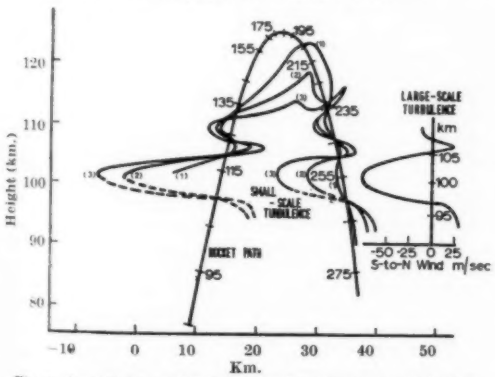

Fig. 2. South-to-north displacement of sodium trail. *Skylark* 11, Woomera, South Australia, December 3, 1958, 19.43 hr. Numbers on rocket path give time in sec. after launching. Sodium trail at (1) +270 sec., (2) + 330 sec., (3) + 390 sec.

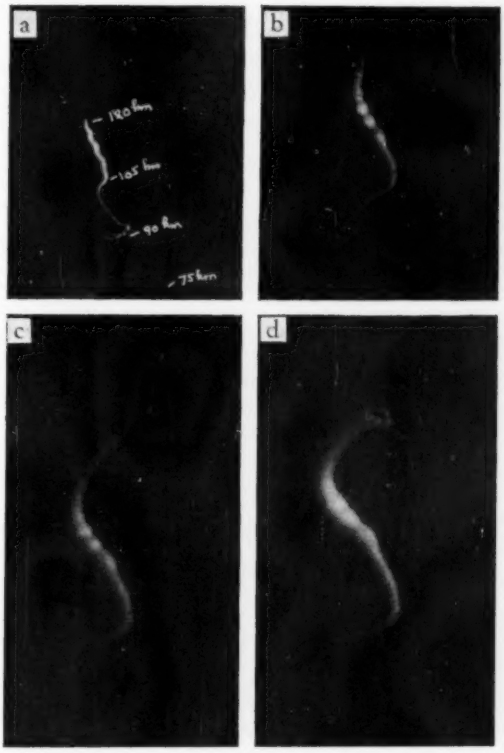

Fig. 3. *a–d*, Exposures of sodium trail made at 2½, 3½, 4½ and 5½ min. after launching

Photography of the trail was undertaken by twelve cameras of either moving-film or fixed-plate design, distributed between three roughly symmetrically placed observation posts. All cameras carried orange filters and were orientated in slightly different directions to increase sky coverage. Exposures were of 5-sec. duration, synchronized to within ½ sec., and were made at intervals of either 1 or 5 min. The trail was bright enough to be recorded photographically for about 10 min., and persisted visually for about 20 min.

The series of photographs reproduced here were taken at 1-min. intervals from + 2½ to + 5½ min. from the time of launch, and the rocket is still in the field of view of the first three. The width of the sodium trail reaches a maximum near the vertex of the trajectory on account of the slower speed of the rocket and the lower ambient air density into which the vapour expands. The short waviness of the trail near the vertex is thought to arise from the rotation of the rocket as the wave-length corresponds closely to the measured spin. The main distortions of the trail arise from the wind structure, as the sodium

atoms very quickly pick up the mean motion of the atmosphere at these heights.

The method of triangulating the trail depends on identifying corresponding features on various photographs taken from different camera posts. The identified points are then measured up in a plate-measuring machine, together with a number of star images as reference points. The processing of the data was carried out at the Admiralty Research Laboratory, Teddington, using the *Pegasus* computer. The accuracy of the triangulation depended on the width of the trail and on the presence of readily identifiable features. The width of the trail increased from about 1 km. at the lower heights to 3 km. at the vertex, and the standard deviation in the triangulation was usually less than half the width of the trail.

Fig. 1 shows the rocket trajectory and the sodium trail projected on the east–west vertical plane. The complete trail is plotted at + 4½, + 5½ and + 6½ min. Fig. 2 shows the corresponding results for the south–north direction. The rocket trajectory was obtained optically using contraves, the data being supplied by W.R.E., Salisbury.

Figs. 1 and 2 show several interesting features. Small-scale turbulence appears to be present to a height of 102 km., while above this level, the trail remained unbroken. At the lower heights the trail broke up increasingly rapidly, and at 94 km. had disappeared within 2 min. Fig. 3*a* shows this part of the trail at about 1 min. after formation. From 94 to 102 km. the trail took some 8 min. to break up.

Below 107 km., the air motion is horizontal, and portions of the trail on both the upward and downward legs of the flight path are correspondingly displaced. The variation of wind speed with height is shown in Figs. 1 and 2 from 93 to 107 km. Above 107 km., the air appears to be moving in large cells with vertical motions of 50 and more m./sec. The cell size is difficult to estimate on the evidence of a single firing, as the trajectory intersects only a small part of the wind structure. The horizontal extent appears from Figs. 1 and 2 to amount to several tens of kilometres.

Acknowledgments are gratefully made to the Royal Aircraft Establishment for provision of the rocket and arranging its firing (particularly Dr. E. B. Dorling); to the Weapons Research Establishment, Australia, which carried out the firing and operated the cameras (particularly Mr. P. Moran and Mr. J. S. Colville); to Dr. E. B. Armstrong and Dr. J. Mazur, The Queen's University, Belfast, who prepared the first trial burners and carried out tests on them; and to the Mathematics Department, Admiralty Research Laboratory, Teddington (Miss M. Thorpe, Mrs. L. Blake) for triangulation computations on the sodium trail.

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FATE OF TRANSFORMING DEOXYRIBONUCLEATE FOLLOWING FIXATION BY TRANSFORMABLE BACTERIA

I

By Dr. MAURICE S. FOX and Dr. ROLLIN D. HOTCHKISS

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An analysis of the kinetics of the reaction between transforming deoxyribonucleate and transformable pneumococci has shown first a reversible adsorption of the deoxyribonucleate by the bacteria, followed by an irreversible fixation of the deoxyribonucleate[1]. Fixed deoxyribonucleate is defined as that which cannot be removed either by exhaustive washing or treatment with deoxyribonuclease. The quantity of such deoxyribonucleate fixed by the transformed population of bacteria is proportional to the number of bacteria transformed; and the efficiency with which such fixed deoxyribonucleate may yield a transformed bacterium is very high[2–4]. For preparations of the most active deoxyribonucleates less than two and perhaps one bacterial equivalent of donor deoxyribonucleate fixed by the recipient population yields a bacterium transformed with respect to a particular genetic property observed.

The experiments to be described represent an attempt to examine the growth of the transforming deoxyribonucleate following its fixation by transformable bacteria. Transforming deoxyribonucleate carrying both a biological label and a phosphorus-32 label has been introduced into transformable bacteria. After various times of incubation following the fixation of the labelled deoxyribonucleate, the total bacterial deoxyribonucleate from aliquots of the transformed population is isolated. This newly isolated deoxyribonucleate is used to transform a second bacterial population and the ratio of its biological activity to the amount of phosphorus-32 fixed by this new population is determined. An increase in the absolute quantity of the deoxyribonucleate capable of conferring the chosen property, as a consequence of synthesis of new deoxyribonucleate by the original transformed bacteria, results in a dilution of the phosphorus-32 label and therefore an increase in this ratio.

Transformable bacteria sensitive to streptomycin are incubated for 15–40 min. at 30° with the deoxyribonucleate labelled with phosphorus-32 isolated from bacteria resistant to streptomycin. Pancreatic deoxyribonuclease (20 μgm./ml.) is added and the mixture transferred to 37° C. The first sample is removed after 5 min., placed in an ice-bath, and further samples are removed at various times as the culture grows. An aliquot is removed to measure the quantity of phosphorus-32 incorporated and the culture is assayed after 2-hr. incubation for the total number of cells transformed, in this case from streptomycin-sensitivity to streptomycin-resistance.

After sampling for a bacterial count (viable and Petroff–Hausser), the iced aliquots are filtered, thoroughly washed and re-suspended in 0·15 *M* sodium chloride, containing 0·05 *M* sodium citrate and 4 mgm./ml. bovine serum albumin fraction V.

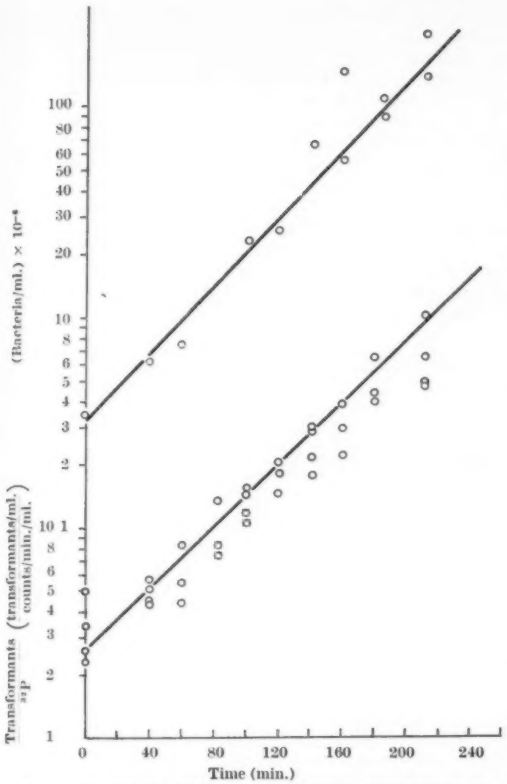

Fig. 1. Growth of bacteria, and dilution of phosphorus-32 of transforming deoxyribonucleate. Transformable bacteria were allowed 25 min. exposure to deoxyribonucleate at 30°. Deoxyribonuclease was added and bacteria were incubated at 37° C. for 5 min. prior to time zero

The re-suspended cells are lysed with sodium deoxycholate at 37° C. (5 min.), precipitated with 1·3 vol. of alcohol, centrifuged, and the crude deoxyribonucleate pellet dissolved in saline buffered with 0·01 *M* phosphate *p*H 7·8. Such solutions are used for transformation of a new culture of streptomycin-sensitive bacteria, measuring number of cells transformed and quantity of phosphorus-32 fixed.

The activity of this re-isolated deoxyribonucleate, when measured in terms of the yield of transformants per unit of phosphorus-32 fixed, is not changed significantly by passage through the bacteria if the deoxyribonucleate is re-isolated 5 min. after fixation (Table 1). In Table 1 are indicated the values of these activities before and after passage through transformable bacteria for a number of deoxyribonucleate preparations labelled with phosphorus-32 of various specific radioactivities. For deoxyribonucleate re-isolated after longer incubation and growth of the transformed bacteria, this activity increases as a function of time (Fig. 1). Also shown in Fig. 1 are the counts made in a Petroff–Hausser chamber of the number of bacteria per ml. The similarity of these two curves suggests that the transforming deoxyribonucleate, or material resembling the transforming deoxyribonucleate, increases at the same rate as do the bacteria in the total culture.

Table 1. Recovery of Phosphorus-32 labelled Deoxyribonucleate (DNA) Activity following Passage through Transformable Bacteria

Duration of exposure to ^{32}P-labelled DNA	Transformants per unit of ^{32}P fixed for original DNA	Transformants per unit of ^{32}P fixed for DNA re-isolated after 5 min. incubation at 37° with DNase
36 min.	8	15
40 min.	6	5·4
25 min.	2·8	3·4
15 min.	100	104
15 min.	28	30

These data are insufficient to indicate whether or not it is possible to superimpose these two curves. In order to determine more precisely the relationship between these two growth-curves, the biological activity of the newly fixed deoxyribonucleate was directly compared with the biological activity of deoxyribonucleate resident in the recipient bacteria.

In a manner similar to that described above, transformable bacteria sensitive to streptomycin and resistant to *p*-nitrobenzoic acid (that is, 'wild type', see ref. 5) were exposed to deoxyribonucleate isolated from bacteria resistant to streptomycin and sensitive to *p*-nitrobenzoic acid. Thus, the deoxyribonucleate responsible for streptomycin-resistance was introduced into bacteria carrying resident deoxyribonucleate responsible for resistance to *p*-nitrobenzoic acid. The process of deoxyribonucleate fixation was terminated after 15 min. at 30° C. by the addition of deoxyribonuclease. The transformed population was transferred to 37° C., 5 min. allowed for action of deoxyribonuclease, and samples, were removed at various times thereafter. Deoxyribonucleate was isolated from the bacteria in these samples and the relative biological activities of the incoming versus the resident deoxyribonucleate were measured. This ratio shown in Table 2 is established at the earliest time of observation, 5 min. after the termination of exposure to deoxyribonucleate, and does not change with the growth of the transformed culture. In addition, the ratio assumes a value approximately equal to the absolute frequency of transformants observed in the first transformation.

Table 2. Relative Biological Activity of Newly Introduced Property to that of a Resident Property following Growth of the Transformed Culture

Ratio of number of cells transformed to total number of cells in transformed culture	Relative increase in turbidity of transformed culture at time of DNA re-isolation	Ratio of biological activities of newly introduced DNA to resident DNA
$6·7 \times 10^{-3}$	(1·0)	$5·2 \times 10^{-3}$
	1·5	$6·7 \times 10^{-3}$
	2·2	$5·4 \times 10^{-3}$
	3·1	$6·1 \times 10^{-3}$
	3·5	$5·4 \times 10^{-3}$
	4·5	$6·0 \times 10^{-3}$
	6·1	$5·5 \times 10^{-3}$
	25·0	$5·5 \times 10^{-3}$

These observations give further evidence of the high efficiency with which fixed transforming deoxyribonucleate may give rise to transformed bacteria. It would appear that transforming deoxyribonucleate re-isolated a short time after fixation has suffered neither physical nor biological dilution, though it is admixed with deoxyribonucleate of the transformable host bacteria. Furthermore, it has been shown that during the growth which follows the fixation of the transforming deoxyribonucleate, this deoxyribonucleate increases at precisely the same rate as does the resident deoxyribonucleate of the transformed bacteria. This synchronization is already established at the earliest times that have been examined in these experiments.

II

By Dr. MAURICE S. FOX

An examination of re-isolated deoxyribonucleate from transformed populations of pneumococci, following moderately long exposures to transforming deoxyribonucleate, has indicated that the new deoxyribonucleate is fully synchronized with the resident deoxyribonucleate of the transformed bacteria from the earliest times that had been observed. In addition, it has been shown that passage of transforming deoxyribonucleate through transformable bacteria does not appreciably alter its biological activity (previous communication).

Further investigation of such re-isolated deoxyribonucleate immediately following short exposures to the transforming deoxyribonucleate has demonstrated an intermediate state of the transforming deoxyribonucleate between its fixation by the bacteria and that state in which the production of transforming deoxyribonucleate is fully synchronized with that of the resident deoxyribonucleate of the transformed population. The deoxyribonucleate in this intermediate state is biologically inactive in transferring in a second transformation the property which it carries. In addition, in line with the segregation investigation being carried out in this laboratory by Hotchkiss, it has been possible to examine the appearance of genetic linkage occurring as the result of an interaction between entities present in the donor deoxyribonucleate and those present in the recipient bacteria, following fixation of such deoxyribonucleate.

The genetic properties that were studied are streptomycin resistance *S*, two readily distinguishable levels of sulphonamide resistance, *a* (fully resistant to 10 μgm. sulphonamide/ml.), and *d* (resistant to 40 μgm. sulphonamide/ml.), and the combined property, *ad* (resistant to 200 μgm. sulphonamide/ml.). Conditions for selection and quantitative measurement of transformed bacteria carrying these properties have been described[5]. The properties *a* and *d* have been shown to be closely linked[5]. Deoxyribonucleate isolated from bacteria carrying both properties yields on transformation, under the conditions used, about as many transformed cells with only the properties *a* or *d* as those carrying the combined property *ad*.

The general procedure followed is similar to that described in the previous communication. After a high-density suspension of transformable bacteria is exposed to deoxyribonucleate for a given time at 30° C., a high concentration of deoxyribonuclease (20 μgm./ml. Worthington, pancreatic) is added to stop any further fixation, and samples are chilled at 0° C. after various incubation times.

After thorough washing, deoxyribonucleate is re-isolated from the bacteria. More than 50 per cent of the newly introduced deoxyribonucleate phosphorus-32 is recovered. Its biological activity is determined by using this deoxyribonucleate to transform a new strain of pneumococcus with respect to one of the properties carried by the original donor deoxyribonucleate and one or more of the properties resident in the recipient bacteria used in the first transformation.

It has been shown in the previous communication that after moderately long exposure to deoxyribonucleate, the ratio of these two activities (newly introduced deoxyribonucleate)/(resident deoxyribonucleate), assumes a value equal to the frequency of transformants in the first transformation, and that this ratio remains constant during the further growth of the transformed population.

It is now found that after very short exposure times to deoxyribonucleate and immediate re-isolation, this ratio is only a very small fraction of the ultimate ratio observed after longer incubation. This ratio rises and becomes a larger and larger fraction of the ultimate ratio with increasing exposure times to deoxyribonucleate (Table 3). Thus, the fractional recovery of the newly introduced deoxyribonucleate increases with increasing exposure times in the first transformation.

Table 3. Recovery of Newly Introduced DNA after Various Exposure Times to Deoxyribonucleate (DNA) at 30°

Time of exposure to DNA (min.)	Frequency of transformants	Ratio of input activity to resident activity for DNA isolated after 1 min. incubation at 37° C. with DNase	Fraction of input activity recovered	Ratio of input activity to resident activity after 2 hr. growth at 37° C.
3	2×10^{-3}	7×10^{-5}	5 per cent	$1{\cdot}5 \times 10^{-3}$
7	5×10^{-3}	10^{-3}	20 per cent	5×10^{-3}
15	$1{\cdot}2 \times 10^{-2}$	$3\ 4 \times 10^{-3}$	31 per cent	$1{\cdot}1 \times 10^{-2}$

The time course of the recovery of the biological activity of the newly fixed deoxyribonucleate has been examined. After very short exposure (5 min.) to the transforming deoxyribonucleate, and re-isolation of deoxyribonucleate from samples of the transformed population that have been allowed increasing incubation times, measurement of the biological activity of such preparations of deoxyribonucleate shows increasing fractional recoveries of the newly introduced deoxyribonucleate. The ratio of the biological activity of the newly introduced deoxyribonucleate to that of the resident material rises to a constant value determined by the frequency of transformants obtained as a consequence of the original 5-min. exposure to the deoxyribonucleate (Fig. 2). This level is identical with that observed after long exposures to deoxyribonucleate (previous communication).

The rates of recovery of the lost biological activity are the same when the newly introduced deoxyribonucleate comes from an organism carrying the properties *S*, *d* or *ad*. In addition, the rates show a dependence on temperature such that at 30° the half-time for recovery is about twice as long as at 37°.

Making use of the possibility of building up the linkage group *ad*[5] from its component parts, a transformable strain of pneumococcus carrying the properties *S* and *a* has been transformed with deoxyribonucleate isolated from a strain carrying the property *d*. The biological activity of the re-isolated deoxyribonucleate is then tested on bacteria carrying none of these properties. The recovery of deoxyribonucleate carrying the newly introduced *d* property follows the time course already described. It is also possible to observe the appearance of a new biological entity that is capable of introducing simultaneously both properties *a* and *d*. The appearance of this new entity, a product of the interaction between the newly introduced and the resident deoxyribonucleates, follows a time course roughly similar, with a half-time about twice as long as that observed for the recovery

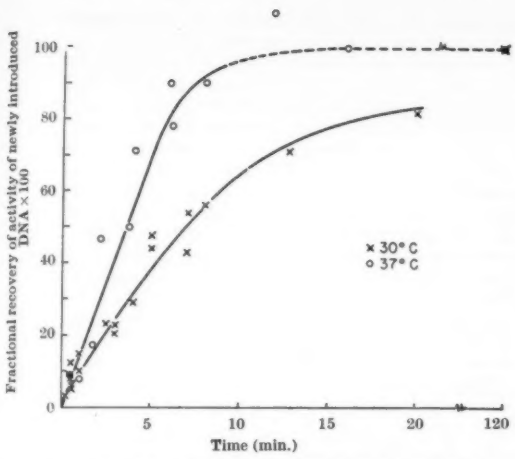

Fig. 2. Fractional recovery in re-isolated deoxyribonucleate of biological activity of newly introduced deoxyribonucleate as a function of time following termination of a 5-min. exposure to transforming deoxyribonucleate with deoxyribonuclease

of the *d* marker that has been introduced (Fig. 3). Thus, the biological activity of the newly introduced deoxyribonucleate recovers with a half-time of about 3–4 min. and the linking of this new deoxyribonucleate with that present in the genome of the recipient cell occurs with a half-time of about 6 min. at 37° C.

The fractional recoveries shown in Figs. 2 and 3 are measured as the ratio of the biological activity of the newly introduced deoxyribonucleate to that of the resident deoxyribonucleate. They are expressed relative to the ultimate ratio observed for deoxyribonucleate isolated after 2-hr. incubation at 37° C. of a dilution of the transformed culture. This incubation is sufficient to allow about a ten-fold increase in the number of bacteria. The ultimate ratio agrees within experimental error with the absolute frequency of bacteria transformed for a single property. For the

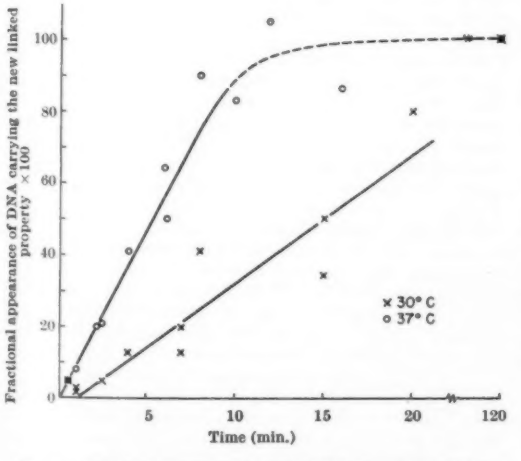

Fig. 3. Fractional appearance, in re-isolated deoxyribonucleate, of biological activity manifesting linkage between the newly introduced and resident deoxyribonucleate. A 5-min. exposure to transforming deoxyribonucleate is terminated with deoxyribonuclease and deoxyribonucleate is re-isolated from bacteria allowed the indicated additional incubation time

cases in which the linkage group *ad* is observed, the ultimate ratio assumes a value equal to one-half the frequency of *ad* transformants in the first transformation. This factor of two difference between the frequency of transformants and the recoverability of the linkage group is to be expected since deoxyribonucleate carrying the *ad* property regularly gives equal numbers of *d* and *ad* transformants.

Thus, there exist at least two processes that transforming deoxyribonucleate may undergo following its fixation by bacteria. One has as its consequence the recovery of the biological activity in re-isolated deoxyribonucleate and the second results in the specific coupling of this newly introduced deoxyribonucleate with that resident in the recipient bacteria. The difference between these two processes may be further examined by a consideration of the relative transforming activities of a given deoxyribonucleate re-isolate with regard to the newly introduced property and the linked property.

Since deoxyribonucleate from bacteria carrying the linkage group *ad* yields on transformation about twice as many transformants bearing *d* (including *ad*) as *ad*, deoxyribonucleate isolated from a transformed population in which about a half of the transformed cells are *d* only and half are *ad* would be expected to yield four times as many *d* (including *ad*) as *ad* transformants. When deoxyribonucleate is re-isolated from a transformed population in which deoxyribonucleate from bacteria carrying the linkage group *ad* is introduced into sulphonamide-sensitive bacteria, the ratio:

$$\frac{\text{yield of } d\text{, including } ad,}{\text{yield of } ad}$$

(d/ad) assumes a value of close to four from very early times (Table 4).

Table 4. Relative Activity of a Single Property, Newly Introduced, compared with a Linked Pair of Properties (*a*) Both Members of the Linkage Group, (*b*) One Member of the Linkage Group, Newly Introduced, and One Member Resident in the Recipient Bacteria

(*a*) DNA from strain *ad* into sulphonamide-sensitive strain

Time at 30° before re-isolation of DNA (min.)	¼	3	7	14	20	120 (at 37° C.)
d / *ad*	3·6	3·4	4·8	4·0	3·9	4·2
Per cent *d* recoverable	4	21	54	70	100	100

(*b*) DNA from strain *d* introduced into strain *Sa*

Time at 30° before re-isolation of DNA (min.)	¼	2½	7	20	20 (at 37° C.)
d / *ad*	13	10	9	6	4
Per cent *d* recoverable	6	14	40	100	100

In the experiment described in Table 4, in which deoxyribonucleate from bacteria carrying the property *d* is introduced into bacteria carrying the property *a*, the ratio *d* : *ad* assumes a high value at early times and approaches an equilibrium value of four at longer incubation times. The fact that the ratio *d* : *ad* is independent of the frequency of bacteria transformed either to *d* or *a* excludes the possibility that any appreciable fraction of the *ad* transformants observed are the consequence of a random double event.

The difference in the behaviour of the *d* : *ad* ratio as a function of time between the two experiments described shows that the recovery of *ad* activity from newly introduced deoxyribonucleate carrying *ad* is a different process from that in which *ad* activity appears as a consequence of an interaction between

the newly introduced deoxyribonucleate and that of the recipient bacteria. It is impossible as yet to distinguish with certainty between a sequential process, recovery of *d*, followed by linking *a* and *d*, and two independent simultaneous processes occurring at different rates.

It is also not as yet possible to say whether or not synthesis of deoxyribonucleate is an obligatory requirement for either the recovery or the integration of the new deoxyribonucleate. Both processes are complete within a period during which there has been little overall growth; the increase in turbidity of the total culture may be less than 10 per cent. Earlier observations have demonstrated that transforming deoxyribonucleate re-isolated after prolonged exposures to deoxyribonucleate, followed by incubation for 5 min. at 37° with deoxyribonuclease, has suffered neither physical nor biological dilution. These times are long enough for both these processes to have approached completion. Furthermore, concentrations of 5-fluorodeoxyuridine thirty times higher than that necessary completely to inhibit bacterial growth fail to inhibit either reaction. Preliminary measurements of incorporation of phosphorus-32 into deoxyribonucleate during this period suggest that the net new synthesis of deoxyribonucleate by such transformed bacteria is less than 5 per cent.

Thus, it may be concluded that transforming deoxyribonucleate becomes integrated into the genome of the recipient bacteria during the first few minutes following the fixation of the deoxyribonucleate. Transforming deoxyribonucleate re-isolated immediately after its fixation has suffered a loss of function, and this function is rapidly recovered on incubation of the transformed bacteria. In addition to a recovery of function, the newly fixed deoxyribonucleate may become genetically linked to deoxyribonucleate resident in transformed bacteria during this period. Preliminary observations suggest that little or perhaps no new deoxyribonucleate synthesis is required for either the recovery of function or the appearance of genetic linkage.

The technical assistance of Miss I. Miller is gratefully acknowledged.

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EFFECT OF ACRIDINE ORANGE ON LIVING BULL SPERMATOZOA

By C. VAN DUIJN, JUN.

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SINCE the introduction of the acridine orange vital fluorochroming technique in 1940[1-3] vital fluorochroming of spermatozoa with this substance was performed for the first time by Strugger and Rosenberger in 1944[4]. Goat spermatozoa showed different resistance to varying concentrations of acridine orange. Using a dilution of 1:80,000 in a glucose-phosphate buffer after Milovanov, 100 per cent motility could be maintained for two days in complete darkness at 8° C. In artificial insemination experiments with fluorochromed semen, fertilization was accomplished in 21 goats out of 27 and this was followed by normal development and birth of young.

Although most living cells do not suffer toxic effects from acridine orange if this is used in very low concentration[3,5,6,7] some cells may be more susceptible. Personally, I found that mouse lymphoblasts are killed by acridine orange in a concentration of 1:5000; even in concentrations as low as 1:50,000 reproduction was impaired[8]. With human spermatozoa, susceptibility was found to be rather high, 1:10,000 being about the highest concentration that may be used without killing them instantaneously, and even then great care has to be taken as to the method of application[9]. Susceptibility also seemed to depend on the activity of the specimens; the more active the spermatozoa, the quicker they were killed.

In recent years Vandemark *et al.*[10,11] have applied acridine orange for evaluating the motility of bull spermatozoa. They did not observe toxic reactions with concentrations of 1:40,000 up to 1:10,000 during 15 min. incubation at 37° C. and they observed only a slight reduction in motility after storage at 5° C. for 24 hr. These estimations, however, were based on visual observations only and not on exact measurements.

Contrary to these observations, I have found a definite adverse effect of acridine orange on living bull spermatozoa, both with respect to the number of normally moving sperms and their mean velocity, measured with high accuracy with the photoelectric method developed by Rikmenspoel[12,13].

Freshly ejaculated semen was allowed to cool to 25° C. and then diluted 10 times with an ultramicroscopically clear egg-yolk–citrate buffer[14] (*p*H 6·75 ± 0·05 at 22–23° C., corresponding to *p*H 6·80 ± 0·05 at 37° C.) of 30° C. After this first dilution the samples were slowly cooled to ~ 4° C. by placing the tubes in a larger glass beaker which in its turn was placed in a Dewar vessel with ice. Further four-fold dilutions were made with portions of the same buffer to which varying amounts of acridine orange had been added to get a range of concentrations of 1:200,000–1:5000. The fluorochrome-containing buffer was poured gently on top of the fluid to avoid mixing, and the sample was allowed to stand in the Dewar vessel for 20 min. before mixing was accomplished by gentle turning; this cautious procedure is required to avoid shocking the specimens when semen is diluted. After mixing, drops (13·5 ± 0·3 μl.) of the diluted semen were brought into a micro-chamber by means of a leukocyte pipette and warmed up for 5 min. to 37° C. Then the dark-field image of individual swimming spermatozoa was projected on to a small aperture 'viewed' by a photomultiplier through an auxiliary lens. Every time a sperm passes by the area 'seen' by the photomultiplier the latter receives light impulses and emits a corresponding number of photo-electrons. The resulting fluctuating photocurrent is amplified and recorded. From the recordings the velocities of individual spermatozoa can be measured, discrimination between normal and abnormal movements is possible and the

method also allows one to determine the total number of normally moving sperms.

The mean velocity of the spermatozoa with normal movement was found to decrease proportionally with the square root of the fluorochrome concentration, according to the equation:

$$\bar{v}_c = \bar{v}_0 - 3900\sqrt{c} \quad \mu/\text{sec.}$$

where $\bar{v}_c$ is mean velocity at fluorochrome concentration c; $\bar{v}_0$ is mean velocity without fluorochrome addition; and c is concentration of acridine orange expressed in weight ratios, for example, $c = 2 \times 10^{-4}$ is equivalent to the expression 1:5,000.

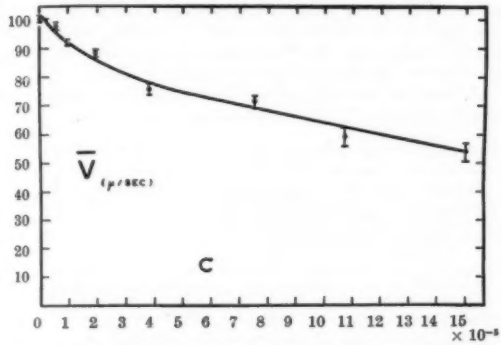

Fig. 1. Mean velocity of normal bull spermatozoa as a function of the concentration of acridine orange. The plotted results have been obtained by pooling all v values of the individual spermatozoa from five ejaculates from four different bulls at each concentration

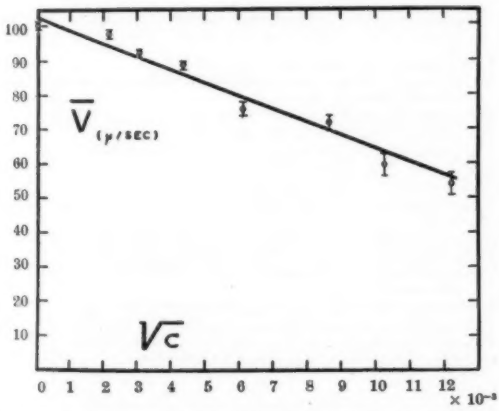

Fig. 2. Mean velocity of normal bull spermatozoa as a function of the square root of the concentration of acridine orange. Same results as depicted in Fig. 1

This relationship is shown by Figs. 1 and 2 representing the data obtained in a series of measurements of 5 ejaculates from 4 different bulls of Dutch-Friesian and red-and-white Meuse-Rhine-Yssel breed. It is shown that the mean velocity at an acridine orange concentration of 1:10,000 has decreased to 62 per cent of that measured in the control without fluorochrome addition under the conditions of the experiment, which include 7 min. irradiation with white light obtained from a 6 V., 5 amp. incandescent microscope bulb, operated at 6 amp. The mean irradiation period is only half this, since the period covers the total time required for recording.

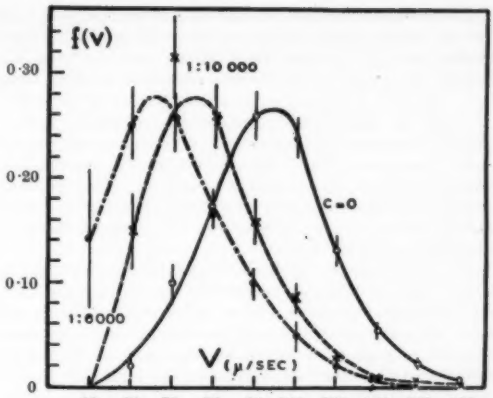

Fig. 3. Normalized velocity frequency distributions of normal bull spermatozoa with acridine orange 1:6,000 and 1:10,000 compared with control ($c = 0$) without fluorochrome

The velocity frequency distributions not only shift to lower values, but their shape is altered, too, as shown in Fig. 3. Whereas at low concentrations the curves do not differ much from that obtained for the control, the distributions become definitely skew at concentrations of 1:10,000 and higher.

The number of normally moving specimens also decreases with increasing fluorochrome addition (Figs. 4 and 5). The effect is not bull-specific, the slopes of the regression lines being identical within the experimental errors for all experiments. Although there

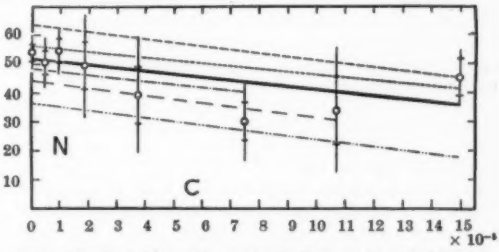

Fig. 4. Number of normally moving bull spermatozoa (arbitrary units) as a function of the concentration of acridine orange. Dotted lines: regression lines of the individual experiments; full line: regression line of all data, with indications of the standard deviations and the standard errors of the means of the individual figures

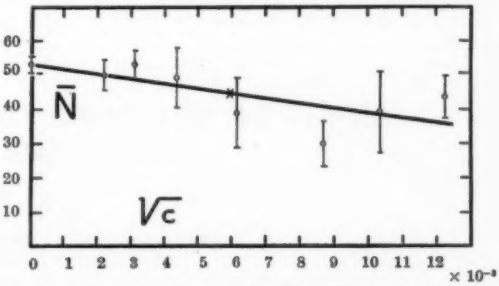

Fig. 5. Number of normally moving bull spermatozoa (arbitrary units) as a function of the square root of the acridine orange concentration. The mean values $\bar{N}$ obtained by averaging the data obtained for the individual ejaculates have been plotted with the standard errors of the means. Comparison with Fig. 4 shows that there is no significant difference in fitting the data to proportionality with either c or $\sqrt{c}$

might be reason to assume that this relationship would also be proportional to the square root of the concentration, the curves show that in this case the data can equally well be fitted by an equation of the type $N_c = N_o - k.c$ as by one where $N_c = N_o - k\sqrt{c}$ (N_c and N_o being the numbers of normally moving sperms, expressed in arbitrary units, at concentration c and 0, respectively). At an acridine orange concentration 1:10,000 the number of spermatozoa showing normal movements is shown to have fallen to approximately 80 per cent of that of the control without fluorochrome addition.

Acridine orange exerts a powerful photodynamic action[5] like all fluorescent dyes[15–17], and in preliminary experiments I have found a great difference in the rates of decrease of velocity with time in illuminated samples of semen to which acridine orange had been added and the controls, consisting of a similar fluorochromed sample kept in the dark and samples without fluorochrome addition kept in darkness and under illumination. Even native bull spermatozoa are highly susceptible to light; Norman and Goldberg[18] found a progressive decline in metabolic activity, followed by the premature death of the cells, showing all characteristics of a photodynamic action, due to the effect of a photosensitizer which is an intrinsic component of the cell. That the adsorption of acridine orange can add to its effect will not be surprising.

Since the technique employed by Vandemark *et al.*[11] implies the use of pure ultra-violet irradiation it seems unlikely that the discrepancy between their results and mine would be due to differences in photosensitizing effects, although this might require additional information on the dependence on wave-length of this effect of acridine orange with respect to spermatozoa. At present it seems more likely that the discrepancy can be accounted for by the low reliability of visual motility estimations in general as compared with exact measurements of velocity.

In recent years it has repeatedly been asserted that acridine orange should be adsorbed preferentially to nucleic acids[6,7,19–25]. Whereas it may be that this applies to certain types of material and special conditions of application[6,20,26] it would lead to serious mistakes if one should take excessive adsorption of acridine orange in itself as a test for nucleic acids. The original investigations by Strugger[5] point to the great diversity of substances that may heavily adsorb this fluorochrome, whereas I demonstrated that in human spermatozoa strong red fluorescence can be obtained after total removal of nucleic acids by extraction with hot trichloroacetic acid[9,27]. Neither in bull nor in human spermatozoa has differentiation between nucleic acid-containing parts (nucleus, mitochondria in the midpiece, and the axial filament of the tail[27]) and the cytoplasmic components been observed in the living state. In bull spermatozoa the entire head appears fluorochromed rather homogeneously, both in the living and the dead state, allowing the fluorochrome method to be used as most reliable for determining biometrical data of the external head shape and its area[28].

Although it would not seem unreasonable to assume that at least part of the adverse effect of acridine orange might be due to adsorption to nucleic acids, this would require experimental corroboration. The mathematical expression of the effect with respect to the swimming velocity is in agreement with a simple adsorptive process following the Freundlich adsorption isotherm, but this is insufficient for further deductions.

A further account of the investigations, which are being continued, will be published elsewhere.

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EFFECT OF GLYCERINE ON THE X-RAY SENSITIVITY OF *SERRATIA MARCESCENS*

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In 1951 Burnett *et al.*[1] reported that many alcohols and glycols, at concentrations in the 0·1–1 *M* range, protected *Escherichia coli B/r* against the lethal effects of irradiation at 2° C. Later Marcovich[2], using *Escherichia coli K*12 (λ), observed similar protection against aerobic irradiation by glycerine and four other similar compounds. Maximally protected organisms had about the sensitivity of anaerobic organisms, and no protection was observed against phage induction which he had reported previously to

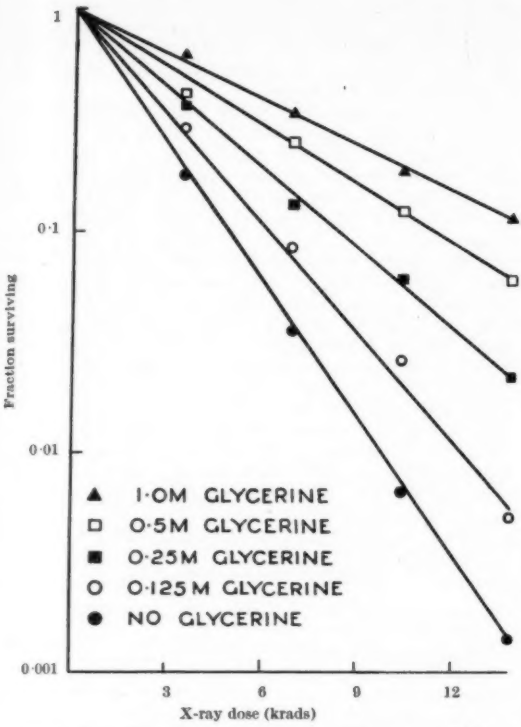

Fig. 1. Logarithmic survival of *Serratia marcescens* suspended in various glycerine concentrations and equilibrated with oxygen during X-irradiation

be independent of the presence of oxygen at the time of irradiation. This phenomenon of radiation protection by glycerine has been re-examined using the small red pigmented bacterium, *Serratia marcescens*, and the observations indicate that protection of aerobic organisms is brought about by some mechanism other than oxygen scavenging or competition with oxygen for radiation-induced radicals, and that organisms which are anaerobic when irradiated can also be substantially protected by the presence of glycerine.

Overnight cultures grown at 25° in unaerated nutrient broth were diluted in 0·015 *M* phosphate buffer at *p*H 7 shortly before irradiation by 200-kV. X-rays (1·6 mm. aluminium filter) at 1,000 r./min. in a 'Perspex' vessel which provided for continuous bubbling and thermostatic control. In the present experiments, the bacteria were diluted and suspended in the glycerine solution a few minutes before irradiation. A control sample was taken just before irradiation, and an experimental sample was taken immediately after irradiation. Both samples were then appropriately diluted in 0·015 *M* buffer containing no glycerine, and plated out in nutrient agar. Visible colony counts were made after 48 hr. incubation at 25°. The fraction of cells surviving to form a visible colony was used as a measure of the X-ray damage.

For anaerobic experiments, nitrogen was bubbled through the bacterial suspension for 20 min. before taking a control sample. Gassing was then continued for a further 5 min. before irradiation in case any atmospheric oxygen had entered the apparatus during sampling. The exit gas was passed through a Hersch cell[3] and any trace of oxygen impurity continuously monitored. Any anaerobic experiments in which the oxygen concentration rose above 20 parts per million have been excluded.

The effect of glycerine on survival of bacteria gassed with 100 per cent oxygen during irradiation is shown in Fig. 1. When plotted as the logarithm of the surviving fraction against linear dose, the points lie on straight lines through the origin at all glycerine concentrations. The slope of the regression line through each set of points has been determined by mean square analysis. The effect of glycerine on the radiosensitivity of bacteria appears to follow the second-order kinetics type of equation used by Michaelis and Menten[4] for enzymes, and Alper and Howard-Flanders[5] for the effect of oxygen on the radiosensitivity of bacteria. If this is so, the effect of glycerine on the sensitivity of bacteria to X-rays can be expressed in the form :

$$\frac{D}{D_0} = \frac{mG + K}{G + K}$$

where D is the 37 per cent dose for the bacteria suspended in a glycerine solution of concentration G, D_0 is the 37 per cent dose in the absence of glycerine. m and k are constants. By plotting D_0/D against $D_0 - D/D \times G$, a straight line should be obtained. By extrapolating this line to cut the axis, values of m and k can be determined. The best line determined by the points from Fig. 1 plotted in this way gives a value for m of 5·25, this being the ratio of the sensitivity of the bacteria in the absence of glycerine to their sensitivity in infinite glycerine concentration. The concentration of glycerine required to give half the theoretical maximum is equal to $K = 0{\cdot}9$ molar. In Fig. 2 the 37 per cent dose has been plotted against glycerine concentration. The solid line is the theoretical curve using the above values of m and k. If glycerine is competing with oxygen for active sites, or preventing oxygen from reaching these active sites then the concentrations of glycerine required to produce a given amount of protection should depend on the concentration of oxygen. The experiment was repeated using a mixture of 1 per cent oxygen in nitrogen in the gas phase. The results shown in Fig. 2 demonstrate that the protective effect of glycerine is independent of a change

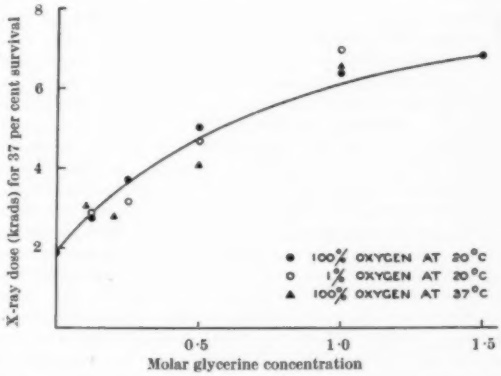

Fig. 2. The effect of glycerine on the aerobic X-ray sensitivity of *Serratia marcescens*

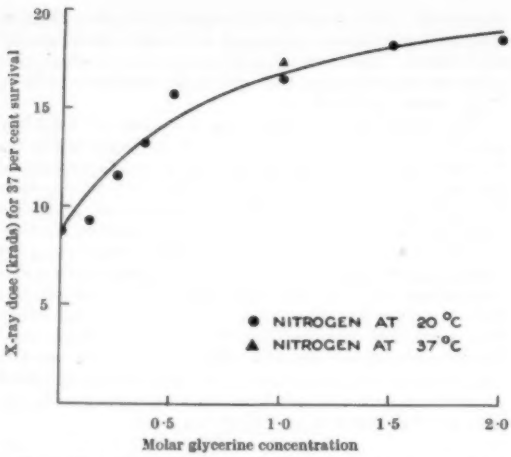

Fig. 3. The effect of glycerine on the anaerobic X-ray sensitivity of *Serratia marcescens*

in oxygen concentration by a factor of 100. Another experiment, using 100 per cent oxygen but at 37°, demonstrates that protection by glycerine has little or no temperature effect. Control bacteria which were suspended in 1·5 *M* glycerine within a minute after they were irradiated, and then kept in glycerine for the same length of time as the experimental bacteria, were not protected against the full X-ray damage.

Glycerine also protected cells from the effect of X-rays when the bacterial suspension was gassed with nitrogen or with 10 per cent nitric oxide in nitrogen anoxia. The kinetics of the effect in the presence of nitric oxide has not yet been worked out. The effect of various glycerine concentrations combined with pure nitrogen is shown in Fig. 3. Using the same procedure as above, the value of m was 2·66 and k was 0·8 molar.

Wood[6] has protected yeast cells against X-ray damage by high concentrations of glycerine. He has interpreted the results as a dehydration of the cells. The overall effects of glycerine on *Serratia marcescens* is very similar to the radioprotection of cysteine on *E. coli* reported by Kohn and Gunter[7]. The effective concentrations of the two chemicals are also very similar. Kohn and Gunter mention the trapping of free radicals or the formation of mixed disulphides as possible protective mechanisms. Burns[8] has been able partially to protect diploid but not haploid yeast against anoxic radiation damage with *p*-chloromercuribenzoate and suggested a masking of sulphydryl groups. No final answer to the mechanism of glycerine protection can yet be given. But, assuming that it protects against the indirect effects of radiation, and extrapolating to infinite glycerine concentration, the dose reduction factor of 2·66 would mean that only 37 per cent of the damage in the anaerobic system was due to direct effects.

I wish to thank Dr. L. H. Gray and Dr. J. W. Boag for helpful discussions, and Mr. J. Steadman and Miss J. Liddon for technical assistance.

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BIOSYNTHESIS OF CELLULOSE BY *ACETOBACTER ACETIGENUM*

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British Rayon Research Association, Heald Green Laboratories, Wythenshawe, Manchester, 22

Morphology of *Acetobacter acetigenum* and Cellulose Synthesis

In the course of a systematic study of cellulose biosynthesis by the bacterium *Acetobacter acetigenum* our attention has turned to the possible function of the cell wall and capsule (that is, a distinct envelope of polysaccharide material) in the production of cellulose. Methods of preparing cell-free extracts of *Acetobacter xylinum* capable of promoting cellulose synthesis have been reported recently. In the Colvin method[1] the extract is prepared from unbroken cells; in other methods[2–4] the extract is prepared from cells which have been disrupted by mechanical or ultrasonic disintegration. An important difference in the behaviour of broken and unbroken cell extracts lies in the ability of the former to incorporate into cellulose the glucose molecule from uridine diphosphate glucose. This observation suggested that it would be advantageous to examine systematically the function of the various cell components in cellulose synthesis. In this article we report that, although *A. acetigenum* is not capsulated, its cell wall plays an important part in cellulose biosynthesis.

Cells of *A. acetigenum* (N.C.I.B. 8132) were grown on standard malt wort[5], and were then inoculated into a sterile medium (*G.M.S.*) of the composition: D-glucose monohydrate, 1 per cent; yeast extract (Oxoid), 0·2 per cent; potassium dihydrogen phosphate, 0·3 per cent; ammonium sulphate, 0·3 per cent; crystalline magnesium sulphate, 0·2 per cent, all by weight in water adjusted to *p*H 6·0. After growth for 40 hr. at 28°, the cells were harvested by the method of Hestrin and Schramm[6]. Microscopic examination of the cells at this stage revealed adherent, ill-defined material surrounding some cells. The cells were then washed three times by suspension in water and centrifugation, and were finally passed through a sintered glass filter (porosity 3). After centrifugation, the precipitated cells were examined by ordinary and phase contrast microscopy in preparations stained by the wet indian ink method[7], dry-film indian ink method[8], and a mordanting procedure[9]; capsules could not be observed surrounding

any of the cells. Washed, filtered cells, when re-inoculated into standard malt wort, rapidly produced cellulose. Repetition of these experiments with *A. acetigenum* cells after growth for 72 hr., 7 and 14 days failed to reveal any capsulation. As a control experiment, well-capsulated cells of *Klebsiella pneumoniæ* were submitted to the same washing and filtration procedures, and distinct capsules were observed under the microscope.

The partial removal of cell walls has been accomplished by the growth of Gram-negative bacteria in the presence of antibiotics[10], or by the partial hydrolysis of cells in the presence of lysozyme and a chelating agent[11]. *A. acetigenum* cells were grown at 28° in the *G.M.S.* medium, to which was added benzyl penicillin (sodium salt) at concentrations of 100, 500 and 1,000 units per ml. For 48 hr. little growth occurred in any of the cultures, and microscopic examination showed many spherical large bodies in addition to normal rod-shaped cells. After this time, slow cellulose formation occurred at the two lower penicillin concentrations, but in no case was synthesis so rapid as in the absence of penicillin. The swelling of cells could not be halted by the addition of polyethylene glycol or sucrose as stabilizers, so that protoplasts could not be isolated.

It was found that cells harvested from 48-hr. *G.M.S.* cultures (without penicillin), and allowed to rest in sterile water at 2° for 2 hr., could be lysed in the following mixture[12]: crystalline egg lysozyme 13·3 μgm./ml.; ethylenediamine tetraacetic acid (sodium salt), 1·4 μmoles/ml.; sucrose, 0·06 m.moles/ml.; 2-amino-2-hydroxymethylpropane-1:3-diol buffer (*p*H 8·5), 33·3 μmoles/ml.; and wet compacted cells, 0·2 ml./ml. After 30 min. at 28°, many protoplasts could be seen under the microscope; more prolonged lysis disrupted the protoplasts giving rise to almost transparent 'ghost' membranes. At all times a few normal, rod-shaped cells remained in the solutions, but differential centrifugation enabled a partial separation to be made. The protoplast-rich fraction, containing very few normal cells, on inoculation into either *G.M.S.* medium or a sucrose solution (*M*) containing mineral salts and yeast extract, did not produce any cellulose for more than 48 hr. at 28°; with normal cells cellulose becomes visible after growth for 3 hr. at 28°. The resumed synthesis with protoplast-containing media may have been due to the recovery of the few normal cells in the inoculum from metabolic upset. However, this partial removal of the cell wall certainly interfered with cellulose synthesis.

We have observed that the growth of *A. acetigenum* in *G.M.S.* medium in both shaken and aerated cultures at 28° produced very little cellulose, and examination showed the presence of large cells in such cultures, similar to the large bodies produced by lysozyme action in the absence of stabilizers. These results have interesting bearings on the findings of Walker and his co-workers[13,14], who observed changes in colonial morphology, and described the cells as 'cellulose-less mutants'. Some doubt has been cast on the purity of such cultures, and it has been suggested that Walker's observations could have arisen from culture contaminants[15]. The formation of protoplasts or even large bodies retards and possibly completely halts cellulose synthesis by such cells, and thus such altered cells could be regarded as cellulose-less mutants. From these observations, it is suggested that the bacterial cell wall plays some part in cellulose biosynthesis.

Extracellular Synthesis of Cellulose by *Acetobacter acetigenum*

THE formation of cellulose by *Acetobacter xylinum* from ultrafiltered ethanol extracts of glucose metabolizing cells has been recorded by Colvin[1]. Similar findings are now reported with *Acetobacter acetigenum*, together with some modifications of the Colvin procedure.

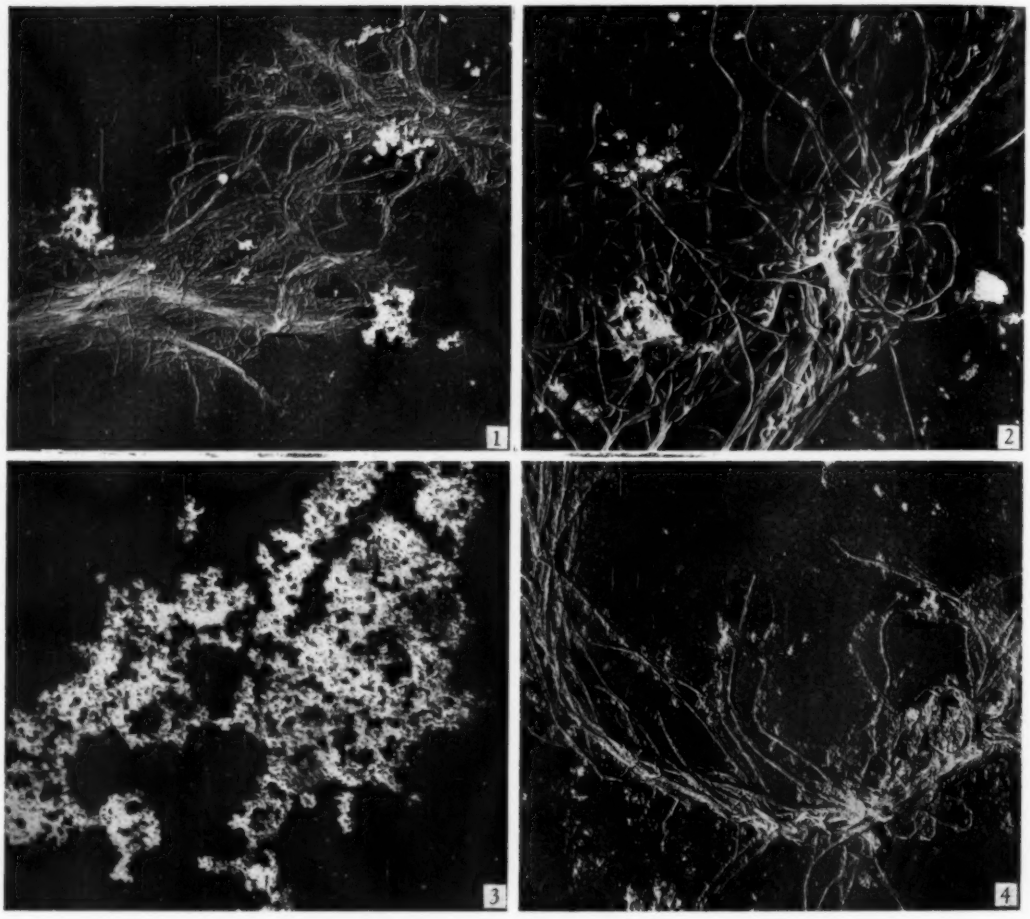
Acetobacter acetigenum (N.C.I.B. 8132) was inoculated from standard malt wort cultures[5] into a medium (*G.M.S.*, see previous communication). The cultures (200 ml. each in 1-l. conical flasks) were incubated at 28° for 40 hr., the cellulose produced then being in the form of an almost transparent gel. Cells were obtained from this gel by the method of Hestrin and Schramm[6]. The cells were washed, incubated with glucose solution, and the precursor of cellulose and the enzyme system were extracted from the cell suspension with ethanol. After removal of cells, the extract was evaporated to give an aqueous solution in which the enzymic reaction with the precursor took place. After evaporation to dryness, the residue was washed with alkali to remove protein and non-cellulosic materials, and then submitted to electron microscopy; all the above operations were carried out exactly as described by Colvin[1].

The resulting electron micrograph (Fig. 1) shows clearly the alkali-resistant, enzymically synthesized microfibrils, although they appear to differ from microfibrils obtained by ultrasonic disintegration of bacterial cellulose (obtained from *A. acetigenum* cultures) and shown in Fig. 2. Acid hydrolysis (2 *N* sulphuric acid for 18 hr. in a sealed tube at 100°) of the alkali-resistant fibrillar residue produced glucose only, when analysed by paper chromatography. Another sample of the ethanol extract was heated in steam at 125° for 20 min. to denature the enzymes present, and then treated as described by Colvin, with the result shown in Fig. 3, revealing no synthesis of cellulose.

Similar alkali-insoluble fibrillar material was obtained by a similar sequence of operations, starting by dropping the gel cellulose into ethanol (to give 75 per cent ethanol concentration, by volume), and then following the Colvin procedure with the ethanol extract.

The efficiency of sinter-glass filters of porosity 5 on 3 for the removal of *A. acetigenum* cells and of microfibrils was tested, in order to examine an alternative method to ethanol extraction. Filtration of an aqueous suspension of cells through such a filter gave a clear filtrate, which contained no cells on examination in the light microscope. Portions of this filtrate were inoculated into standard malt wort[16], and were incubated at 28° for 7 days; no cellulose was formed and no cells could be detected. Thus the filter removed cells completely. Bacterial cellulose was disintegrated ultrasonically (9 kc./s. for 1 hr. at 2°) in water to give microfibrils visible under the electron microscope. Filtration of this suspension through the glass filter, followed by centrifugation of the filtrate at $30 \times 10^3 g$, gave no visible residue, and no microfibrils could be observed in either the supernatant liquid or in washings from the centrifuge tube under the electron microscope. Thus the glass filter could be used to remove microfibrils from solution.

A *G.M.S.* culture (200 ml.), grown for 40 hr. at 28°, was passed through the glass filter and the filtrate was incubated for 1 hr. at 28°. The filtrate was then centrifuged at $30 \times 10^3 g$, and the pre-



Figs. 1–4. Electron micrographs of cellulose microfibrils (× *c.* 13,500) and of denatured enzyme experiment

cipitate was submitted to electron microscopy, with the result shown in Fig. 4; again clear evidence for the formation of microfibrils was obtained. These microfibrils were insoluble in sodium hydroxide solution (2 *N* at 100° for 5 min.), and again their appearance differed from both the samples obtained above (Figs. 1 and 2). The reasons for these differences are not yet known. The addition of sterile aqueous solutions of α-D-glucose-1-phosphate, uridine diphosphate glucose (sodium salt), cellotetraose and cellohexaose (2 mgm. each in 2 ml. water) separately to the ultrafiltered culture medium gave no visible quantity of cellulose, as would have been expected from enzymic synthesis if these substances were extracellular precursors of cellulose.

These experiments confirm the findings of Colvin[1] that the final stage (or stages) of cellulose biosynthesis by *Acetobacter* spp. occurs extracellularly, and also lend weight to the findings of Schramm, Gromet and Hestrin[16] that glucose-1-phosphates and uridine diphosphate glucose provided exogenously are not converted to cellulose on the outer surface of *A. xylinum* cells. All these findings support the hypothesis that an exogenous substrate (probably glucose) enters the cell, and is converted to a precursor which can be transported out of the intact cell and converted to cellulose extracellularly.

We wish to acknowledge the help and advice of Drs. J. Dlugosz and A. Sharples, and the practical assistance of Miss D. L. Peat and Mr. C. D. Shorey. This work forms part of the fundamental research programme of the British Rayon Research Association.

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LETTERS TO THE EDITORS

PHYSICS

Electromagnetic Standing Waves and Ball Lightning

By now it is well known[1-3] that charged particles moving in harmonically oscillating vacuum electromagnetic fields can be subject to time-average forces which establish a virtual potential minimum for them. The matter takes on a different aspect when the number of particles is so large as to form a highly conducting plasma, as in Kapitza's theory of ball lightning[4]. Then, quite aside from the details of the particle motions, we know that the radiation pressure of the inflowing electromagnetic radiation is exerted on the energy-dissipating plasma. Thus, if the maintenance of a local ball of fire requires about 20 kW. (2×10^{11} dyne cm. sec.$^{-1}$), the total force, irrespective of direction, associated with this energy conversion will be 6·7 dynes. To the extent that, besides the 20 kW. absorbed, there may be energy reflected, this number will be greater. A reflected 20 kW. will contribute 13·3 dynes. This is far too little to hold a fire-ball at several thousands of degrees temperature and, say, 10 cm. in diameter from rising under the unidirectional buoyant force of about 600 dynes.

The stability is far more likely to be fictitious, in that the discharge simply stays where the energy is available. This is what happens with a not-too-long horizontal arc in air. Relative to the fresh air moving upward into the arc (because the hot gas in the arc rises) the ionization front moves downward because the power comes from the fixed electrodes. The fireball can be expected to stay in that place relative to the Kapitza standing-wave pattern where the impedance match is best.

This raises a minor difficulty, because the ionization will start at an antinode of the electric field, but the fireball, being of low impedance compared to free space, will lie at a node. Somehow the shift has to be made and the electrical geometry of the space changed without destroying the standing-wave pattern.

A more serious difficulty for the Kapitza theory lies in the magnitude of the power required. This must be about the same as for an electric arc in air or nitrogen at one atmosphere pressure. Analysis of Cobine's Fig. 9·19 (ref. 5) gives as the relation of voltage gradient E to arc current I:

$$E = 82\, I^{-0.57} \text{ (V./cm., amp.)}$$

His Table 9·12 gives a current density of 6 amp./cm.^{2}. The surface area S of the arc per cm. of arc length therefore increases only as $\sqrt{I}$, in fact:

$$S = 1{\cdot}45\, I^{0.5} \text{ (cm., amp.)}$$

Thus, the power dissipation per unit surface area is:

$$\frac{EI}{S} = 57\, I^{-0.07} \approx 57 \text{ watts/cm.}^2$$

Application of this to a fireball 10 cm. in diameter, presumably maintained by a 40-cm. Kapitza wave, yields a required power of 18 kW.

Suppose Nature to be an obliging wench faced now by the necessity of substantiating at least some of the all-too-human accounts of ball lightning; How could she proceed? First, she would recognize that she cannot use St. Elmo's fire as a stand-in, for we have learned to discriminate. Second, since the onset of the phenomenon must only alter the wave pattern a little and not react strongly on the power source, she must have about four times 18, or 72 kW., as a cushion. She will, therefore, have to generate about 90 kW. of short-wave power. At least twice this amount of primary power will be required. Third, to conform to some eye-witness testimony regarding the distance that lightning balls can be from other objects, she will have to be able to maintain the ball at a distance of some 400 cm., more or less, from conducting objects. This is ten 40-cm. wavelengths, so that the frequency will have to be constant to a fraction of 10 per cent, say to 2 per cent. Of course she could arrange for a general background of powerful short-wave radio noise which, augmented locally by a weaker Kapitza standing-wave, could feed a fireball, but the over-all energy requirements would be greater.

She would have to design the wave-guide-system reflectors and lenses, which would focus the energy into a small volume at some distant (4 m.) point, and she would have to buy the klystrons or magnetrons and power amplifiers to put out 90 kW. for some 20 sec. of continuous operation.

This is not beyond our own capabilities, and we have the advantage of more suitable energy sources. A lightning stroke may discharge about 20 coulombs at 5×10^8 V. (ref. 6)—an energy of 10^4 MW.sec. In 20 sec. the fireball itself consumes only 0·36 MW.sec. The capacitance associated with the store of lightning energy is $3{\cdot}6 \times 10^4$ cm., which would be the radius of an isolated conducting sphere bearing the 20 coulombs. This is a reasonable size for a cloud. Actually, the charge forms a dipole in the cloud, but we shall probably not make an order-of-magnitude error if we calculate the energy density as if the energy were uniformly distributed through a sphere of $3{\cdot}6 \times 10^4$ cm. radius. We thus find $5{\cdot}1 \times 10^{-11}$ MW.sec. cm.$^{-3}$. In the fireball it is $6{\cdot}8 \times 10^{-4}$ MW.sec. cm.$^{-3}$, that is, the energy is $1{\cdot}3 \times 10^7$ times as concentrated. Nature does not surpass this degree of augmentation even in the return lightning stroke, despite its being catastrophic. For her to do it over an interval of 20 sec. is another matter.

It seems highly probable that with 90 kW. of 40-cm. continuous-wave power a globular hot plasma floating in air can be formed and maintained by suitably focusing the radiation. Something along this line may, it seems, be contemplated[3], although it is to be noted that 50 kW. is the maximum power of commercial radio broadcasting stations in the United States. Some advantage could be gained by trying for a smaller fireball. A successful experiment will not, however, demonstrate the existence of Nature's own ball lightning. It would only show

that, as in many cases, Nature, as both co-operator and frustrator, permits man to do it.

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Absorption of Ultra-violet and Visible Light by Ultra-thin Sections of Vitrinite from a High-volatile Bituminous Coal

It is a commonly held view that coals contain aromatic structures and that they become more graphite-like with increase in rank. X-ray studies have tended to confirm this concept; however, recent work[1] has shown that small (about 100 atoms or less) aromatic and tetrahedral structures that have comparable numbers of atoms give rise to overlapping X-ray diffraction patterns in the angular regions of the (10), (11) and (20) reflexions of graphite-like layers. The question of distinguishing between the two types of structures will have to be resolved eventually by rigorous and more detailed X-ray or electron diffraction studies. Knowledge of other physical and chemical properties would be of great help in such structural analyses. It has been reported[2] that the extinction coefficients of a high-volatile *A* bituminous vitrinite in ultra-violet and visible light are too low to admit of an appreciable content of condensed aromatic structures. A detailed study of absorption became possible when we succeeded in cutting ultra-thin sections of coals of various ranks and in accurately measuring their thickness and refractive index by interferometry[3].

The ultra-thin sections of vitrinites were obtained by cutting on a microtome, using a diamond knife. The fragments were very small; in most instances the areas suitable for accurate absorption measurements were less than 800μ in diameter. Consequently, a very intense source of light, a 1,000-watt mercury-xenon arc lamp, was used. The light was passed through a grating monochromator and then a prism monochromator to obtain high purity before passing through the specimen. This was found to be necessary to minimize the effect of fluorescence. After traversing the sample, the beam was further monochromatized by reflexion from a precision grating before intensity measurements were made by a multiplier phototube sensitive in the ultra-violet and visible.

Because of the fluctuating brightness pattern of the lamp, it was found necessary to compare the transmission through the specimen mounted on an aperture with that of an identical aperture placed in the same position. Before mounting the specimen, transmission of the two apertures was compared throughout the spectral range of interest at different slit openings to assure that they were placed in the same position. The ultra-thin specimen, mounted on a quartz plate, was arranged over one aperture, using a microscope, and an equivalent clear quartz plate fastened over the reference aperture. The quartz plate in front of the specimen reduced reflexion losses. Relative transmission readings of the two mounts in the same position were then made at the different wave-length settings. In choosing the latter, emission peaks of the lamp above the continuum were used where possible so that minimum slit openings, as low as 0·001 mm., corresponding to a resolution of 0·02 Å., could be used. For readings in the continuum, slit openings ranged from 0·01 to 0·1 mm., usually less than 0·05 mm. Transmission of the reference aperture was measured before and after that of the specimen.

Following the absorption measurements, the thickness of the specimen was measured with an *AO* Baker interference microscope. Phase retardations were measured for the specimen immersed in both air and an immersion oil to permit simultaneous determinations of refractive index and thickness. However, these parameters are not readily calculated for absorbing materials by the interference microscope. The extinction coefficient k, refractive index n, and thickness t are all involved in the solution of the phase-change equation on transmission through an absorbing material; the product kt is obtained from the absorption measurements after slight corrections for reflexion. The calculations are made by a trial-and-error procedure or graphically. This technique will be more fully described elsewhere.

Spectral absorption results have been completed for vitrinite from Pittsburgh bed high-volatile *A* bituminous coal. The thickness of the ultra-thin section used was 550 ± 20 Å., and the refractive index at 5460 Å. was 1·80. The latter value compares favourably with 1·78 found by reflectance measurements on polished blocks[4]. Variations of the specific extinction coefficient of Pittsburgh vitrinite with wave-length are shown in Fig. 1. The coefficient is defined by $K = 10^5 (\log I_0/I)/td$, where the intensity falls from I_0 to I after traversing thickness t (Å.), and d is the density (gm./cm.^{3}). The extinction coefficient, a more fundamental measure of absorp-

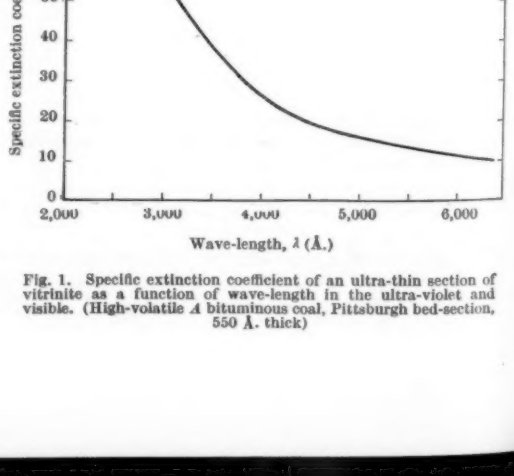

Fig. 1. Specific extinction coefficient of an ultra-thin section of vitrinite as a function of wave-length in the ultra-violet and visible. (High-volatile *A* bituminous coal, Pittsburgh bed-section, 550 Å. thick)

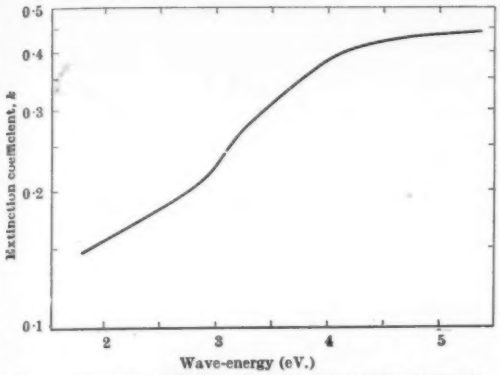

Fig. 2. Extinction-coefficient of an ultra-thin section of vitrinite as a function of wave-energy in the ultra-violet and visible. (High-volatile *A* bituminous coal, Pittsburgh bed-section, 550 Å. thick)

tion, is defined at wave-length λ_0 by $k = \lambda_0 \ln(I_0/I)/4\pi t$, and is equal to the product of refractive index and absorption index. Extinction coefficients for the Pittsburgh vitrinite are shown in Fig. 2 as a function of wave-energy and wave-length.

Ultra-violet and visible light cause the excitation of π electrons and free electron pairs. The electronic excitation is usually accompanied by the excitation of vibrational transitions, and hence absorption bands instead of lines are obtained when solids are examined. Figs. 1 and 2 show that the Pittsburgh vitrinite absorbs a considerable amount of light in the ultra-violet and visible regions. The absorption observed here is larger than that reported for a pyridine extract (in pyridine solution) of the same vitrinite[5]. The larger absorption appears to be reasonable when it is considered that the extract contained more hydrogen, and probably was composed of less-complex molecules than the vitrinite itself. Comparison of the above absorption with those of aromatic compounds indicates that the vitrinite studied contained major amounts of condensed aromatic structures.

High absorption by this coal in the visible region seems noteworthy. It may be caused by presence of structures similar to highly conjugated compounds containing oxygen or to polymerization of polycyclic hydrocarbon structures of 1–5 condensed rings. For example, rubicene, which may be regarded as a polymer of two benzene and one anthracene molecules, absorbs intensely in the red, indeed more than dinaphthocoronene, a ten-ring compound. The long wave-length edge of absorption, denoted by $A_{1g} \rightarrow B_{2u}$ in group theory notation, occurs at 2600 Å. in benzene and shifts progressively toward the red as the number of rings in the molecule increases[6].

The extinction coefficients obtained by Friedel and Queiser[2] for the Pittsburgh vitrinite are 3–4 times lower than those found here. These workers used hand-ground sections probably 3–5-μ thick on the average. We have attempted to prepare ground sections a few microns thick that would be suitable for accurate absorption measurements, but so far have been highly uncertain of effective thickness owing to the presence of very thin areas, scratches or holes in such sections. The possibility was considered that a 'skin' effect resulting from oxidation of the surfaces cut might exist and result in increased absorption. Identical extinction coefficients were obtained when another section of this coal 1060 Å. thick was employed. Furthermore, for other coals, thicknesses ranging from 500 to 1500 Å. had no influence on extinction coefficients, and identical refractive indexes were obtained for 500–1500 Å. and 1–2-μ thick sections. Therefore, it appears that such a 'skin' effect is not significant.

The present absorption results are not inconsistent with earlier X-ray diffraction results[7]. When analysed on the basis that the diffraction peaks at high angles were produced by two-dimensional reflexions of aromatic structures, the X-ray patterns showed that the average layer size was 7·0 Å., corresponding to 3–5 condensed rings. However, vacuum distillation of this vitrinite produced a distillate and residue showing X-ray patterns corresponding to a layer size of 5·8 Å. (1–3 rings)[7]. If the larger layers of the untreated vitrinite result from coplanar coalescence of smaller units or consist of imperfect larger aromatic structures, this vitrinite may be considered a polymer of small, partially oriented condensed aromatic units. However, an authentic analysis of aromatic and tetrahedral structures in coal will await more rigorous and detailed X-ray studies.

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Dependence on Temperature of the Interlayer Spacing in Carbons of Different Graphitic Perfection

IN a previous communication[1], attention was directed to important inconsistencies and gaps in the literature concerning thermal expansion in graphitic carbons perpendicular to the hexagonal layer-planes. Preliminary experiments were reported, and, while these clarified the picture to some extent, the need for further experiments at higher (above 1,200° C.) and lower (below 20° C.) temperatures was indicated. Special diffractometer techniques required for these measurements have now been developed and will be described elsewhere: meanwhile, the extended results are reported.

In Fig. 1*a* measurements made at temperatures up to 2,600° C. are given for a material having a p value (the proportion of disoriented layers) of 0·2. This now clearly establishes a non-linear relationship between interlayer-spacing d (Å.) and temperature t (° C.): the empirical equation over the range 0°–2,600° C. (for $p = 0{\cdot}2$) is $d = 3{\cdot}357 + 91{\cdot}9 \times 10^{-6}\, t + 5{\cdot}3 \times 10^{-9}\, t^2$. For $p = 0$ and $p = 0{\cdot}7_5$, measurements[1] up to 1,188° C. indicate essentially similar curves translated parallel to the temperature axis, and it would seem that while the rate of expansion is dependent on the interlayer spacing it is not signifi-

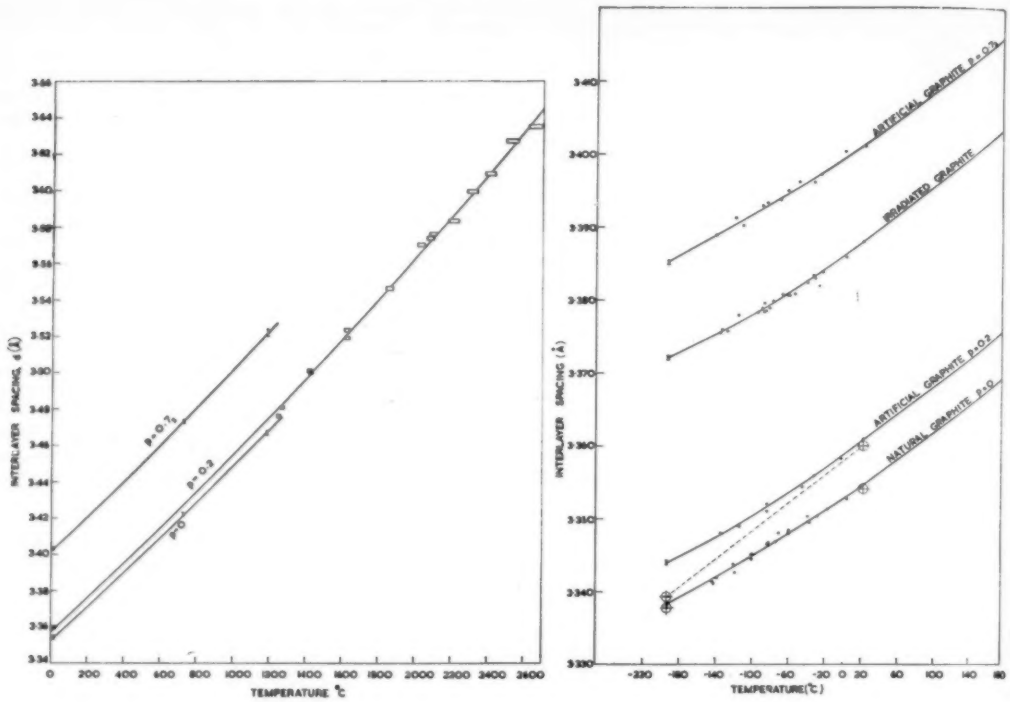

Fig. 1. Interlayer spacings at (*a*) high and (*b*) low temperatures. ⊕ denotes points due to Baskin and Meyer (ref. 3)

cantly dependent, for a given spacing, on stacking disorder.

In Fig. 1*b* measurements at temperatures down to −196° C. for materials having $p = 0$, $0{\cdot}2$ and $0{\cdot}7_s$ are shown. Also included are results for neutron-irradiated graphite having $p = 0{\cdot}2$, $d = d_1 = 3{\cdot}358$ (20° C.) Å. before irradiation, and $d = d_2 = 3{\cdot}388$ (20° C.) Å. after irradiation: with this material the small amount of irradiation produced little or no change in p as assessed from modulation of hk bands, while interstitials resulted in the increase in the average interlayer spacing[2]. The results in Fig. 1*b* give experimental support to the suggestion made previously[1] that for carbons of different graphitic perfection there will be different limiting distances of closest approach between the layers, with decreasing temperature. For $p = 0$, while the interlayer spacing at −196° C. agrees with that reported by Baskin and Meyer[3], the present results show the expected[1] gradual 'levelling-off' rather than the abrupt change in slope implied by those investigators: for $p = 0{\cdot}2$ a 'levelling-off' is again in evidence and the limiting spacing appears to be larger than that found by Baskin and Meyer. Results for $p = 0{\cdot}7_s$ are seen to be consistent with the general picture. With *n*-irradiated graphite, the results are interesting in that the interstitials, which have caused a certain increase in interlayer spacing (from d_1 to d_2) without deterioration in perfection of layer-stacking ($p = 0{\cdot}2$), have had essentially the same effect on the low-temperature behaviour as the greater stacking disorder of an unirradiated less-graphitic carbon ($p = 0{\cdot}6$) having the larger interlayer spacing (d_2).

Consideration of the family of curves for varying perfection over the extended temperature-range (−196° to 2,600° C.) shows that, while the high-temperature behaviour is controlled mainly by the interlayer spacing alone, low-temperature rate of expansion for a given spacing becomes dependent on imperfections and stacking disorder effects.

By adding a term to the quadratic equation given above for the range 0–2,600° C., it is possible to describe both the low- and high-temperature data by a single expression. For example, a term of the type $\frac{A}{(t+B)^n}$, with n a reasonably large number, gives the 'levelling-off' at low temperature and leaves the high-temperature behaviour still essentially described by the original quadratic. Curves for different p values are then derived by using the known (room-temperature) d–p relationships[4].

Further work should include consideration of the lattice dynamics.

We are pleased to acknowledge the assistance of Mr. M. W. Taylor in obtaining these results.

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RADIOPHYSICS

Ionospheric Information from Satellite Signals

THE observation of radio signals from Earth satellites has opened up a new field of investigation for the examination of the ionosphere, particularly above the height of maximum ion concentration. The simplest features to observe are the fading-rate of signals resulting from the different phase paths of the ordinary and extraordinary rays, and the Doppler frequency shift.

To derive ionospheric information from a record of a single satellite transit requires very accurate observations and rather involved calculations, and the results derived in this way confirm the notorious variability of the ionosphere in both time and space.

Where an adequate series of observations is available, however, much useful information can be obtained very simply from the fading-rate. This rate, often referred to, rather loosely, as the 'Faraday fading' rate, is essentially a measure of the total ionization in the path from satellite to receiver. If a mean value is derived at the time of nearest approach, most of the minor variations are sufficiently smoothed out and even the transverse propagation dip[1] can be neglected except in low latitudes. The rate is not critical, as the Doppler shift is, of the closeness of the transit to overhead, so that a series of records over a few days can be used to obtain mean values. Moreover, in a consistent series of observations, it is possible to obtain a significant fading-rate even when much of the pattern is distorted by irregularities and diffuseness which, in themselves, are useful indications of ionospheric anomalies.

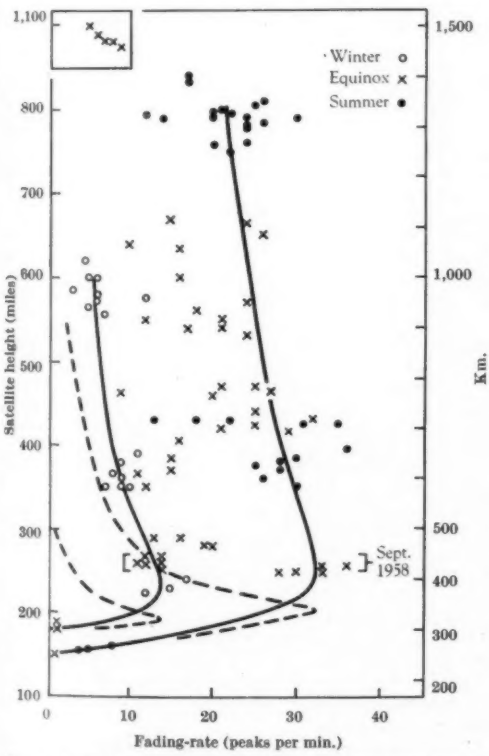

Fig. 1. Satellite signal fading-rates, June 1958–July 1960, 0100–0500

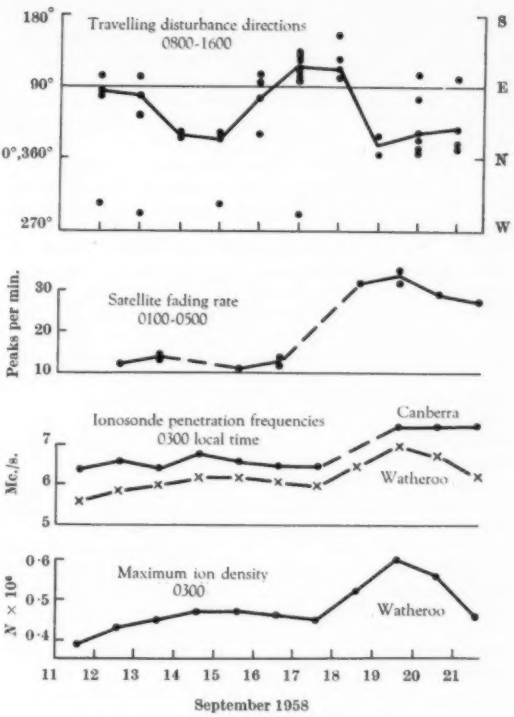

Fig. 2. Ionization changes during September 1958

It is thus possible from regular observations at different times and heights to build up mean 'fading-rate profiles' for any particular observing site. These can then readily be converted to ionization profiles by comparison with corresponding ionosonde recordings, and deviations and their causes can then be studied.

Results derived in this way from two years observations of signals in the 20 Mc./s. band from the satellites *Sputnik III* (1958 Delta 1), *Explorer VII* (1959 Iota 1) and *Sputnik IV* (1960 Epsilon 1) are shown in Fig. 1 plotted as fades per minute against satellite height. Nearest approach times were obtained from Doppler shift records taken simultaneously by a method previously described[2]. Each point plotted represents the mean rate for a particular transit. These observations are for the period 0100–0500 local time when no direct solar influence is present and the decay in ionization is slow. The most obvious feature is the difference between the summer and winter distributions, and different symbols have accordingly been used for summer (November–January), winter (May–July) and the other months classed as equinoctial. Mean fading-rate curves are shown as full lines and the corresponding ionization profiles for summer and winter derived from them graphically in arbitrary units are indicated as dotted lines. The ratio of the summer and winter maxima of ionization is in good agreement with that obtained from ionosonde data.

The features so far mentioned will be dealt with in detail in future papers, and the main purpose of this communication is to report the remarkable result in September 1958 when the rate increased practically from winter to summer values within two days when the satellite was at a height of approximately 400 km.

An abrupt seasonal change in the ionosphere at this time of the year has been known for many years and has been consistently observed in travelling disturbance studies[2]. An examination of the available records for September 1958 did indeed show a marked change in direction of movement at this time, and also a correspondingly marked rise in critical frequency at Australian ionosonde stations. These results are shown in Fig. 2. There are some gaps in the data, but the conclusion is clear. It appears that the directional change occurred first, followed a day later by the change in rate, with the critical frequency change becoming obvious a day later again. Some fluctuation in direction after the main seasonal change is usual.

This sequence suggests a major change in the movement of ionization at this epoch which appears to be consistent with a reversal of the north–south seasonal component of drift indicated by travelling disturbances. Since this change occurred during the Geophysical Year, examination of records at other sites should yield interesting information. A magnetic storm of world-wide incidence but short duration occurred about this time, and its relation to the general change is being examined.

These results indicate the value of simple satellite observations, as a supplement to ionosonde recordings, for ionospheric studies and predictions. The 20 Mc./s. frequency band seems very satisfactory and an orbit such as that of *Explorer VII* is particularly suitable. Its height-range is 350–670 miles and this is swept through every 50 days in each transit at any site. The type of transmission used—continuous-wave with frequency modulation—is also satisfactory. The pulsed transmissions from *Sputnik III* made counting difficult at high rates, but short closely spaced pulses, as used in part of the coding of *Sputnik IV*, would be satisfactory and would enable study of multiple paths. Occasional breaks would be useful also for this purpose and for comparison of spaced receiver records. Telemetry information could also be conveyed as at present.

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Observations of 'Whistlers' and 'Chorus' at the South Pole

Whistlers and chorus are now being observed at the South Geographic Pole (79° geomagnetic), and the seasonal and diurnal variations in occurrence of these phenomena at this unique location follow interesting and perhaps significant patterns.

Maximum chorus activity occurred during the midsummer period, gradually diminished through February and March, and virtually disappeared when the Sun dropped below the horizon. Despite this seasonal dependence on solar zenith angle, there was a surprising and considerable diurnal variation in both strength and occurrence of chorus with a consistent maximum between 1500 and 1600 u.t. corresponding to approximately 1000 hr. local magnetic time.

No whistlers were apparent during the summer period, and it was not until March 8 that the first whistler was heard. Whistlers became quite prevalent after sunset, and the rate of occurrence increased through April and May to reach a maximum during the winter solstice. A considerable and consistent diurnal variation is apparent with maximum activity centred about 0630 u.t.

Although detailed characteristics of these polar whistlers cannot be determined until the tape recordings are available for spectrographic analyses, aural monitoring indicates that they generally resemble whistlers at lower latitudes. Pure tones, swishes and multiples are most common. Echo trains with about $2\frac{1}{2}$ sec. delay between events were heard on May 10 and May 31. The rate of occurrence at Pole Station (averaging 2–3 per min. during active periods) is comparable with typical whistler-rates at middle-latitude stations.

Judging from the observed characteristics of these polar whistlers, it appears that they could have been produced at lower latitudes, reaching the receiver by reflexion between the Earth and lower ionosphere[1,2]. The seasonal and diurnal variations in the occurrence of whistlers at the South Pole may therefore be due to the seasonal and diurnal variations in *D* region absorption over the path of propagation. Assuming that the whistlers are propagated more readily along the path of least absorption, the diurnal peak at 0600 u.t. at Pole Station suggests that the whistlers penetrate the ionosphere somewhere along the 70° meridian, probably in the vicinity of Graham Land, approximately 55° south geomagnetic latitude. In middle latitudes whistler-rates are generally higher at night and in local winter, and therefore consistent with the hypothesis that *D* region absorption affects whistler propagation. If this interpretation is correct, coincidence should exist between whistler occurrences at South Pole Station and Port Lockroy. This will be investigated when the recordings from both stations are available for analyses.

The origin, or origins, of chorus are not fully understood. The seasonal variation of this phenomenon at the South Pole, with maximum activity occurring in midsummer, suggests direct dependence on solar radiation. It seems probable, therefore, that the polar chorus signals are initiated by solar corpuscular radiation impinging on the outer ionosphere.

The association between very-low-frequency hiss and aurora observed at Byrd Station, Antarctica, is also apparent at Pole Station[3]. However, whereas the auroral hiss observed at Byrd Station usually centres about a frequency of 8 kc./s., at Pole Station, 10° higher in geomagnetic latitude, the auroral hiss centres about 9·5 kc./s. This change in frequency of auroræ-associated hiss with latitude is interesting. At 42° south geomagnetic Ellis[4] has associated very-low-frequency noise at 4–6 kc./s. with auroræ, and current investigations also indicate that the frequency of auroræ-associated hiss may decrease with geomagnetic latitude.

The very-low-frequency observations at Pole Station are being made by Oliver C. Morse of the United States Antarctic Research Program. The

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GEOLOGY

Olivine-Spinel Transition on Nickel Orthosilicate

THE pressure-induced transition of olivine to a denser spinel structure is probably of considerable importance in the Earth's mantle[1]. Olivine–spinel transitions are known in Mg_2GeO_4 (refs. 2 and 3) and Fe_2SiO_4 (ref. 1), while a variety of evidence indicates that Mg_2SiO_4 will undergo a similar transition around 125,000 atmospheres and 600° C. (refs. 1 and 3). These results suggested the desirability of exploring for olivine–spinel transitions in other substances which normally crystallize with an olivine structure.

Such a substance is nickel orthosilicate—Ni_2SiO_4. From preliminary[1] and recent (Ringwood, A. E., unpublished results) data on the solid solubility of Ni_2SiO_4 in Ni_2GeO_4 (spinel) and vice versa, I have calculated that Ni_2SiO_4 should undergo an olivine–spinel transition around 54,000 atmospheres at 1,500° C. However, Wentorf[4] has recently reported unsuccessful attempts to synthesize a spinel modification of Ni_2SiO_4 at pressures up to 110,000 atmospheres. I have carried out further high-pressure experiments using a uniaxial pressure apparatus (squeezer), in an attempt to resolve the conflict, and provide further information on olivine–spinel transitions.

Intimate mixtures of $Ni(OH)_2$ and silicic acid in orthosilicate ratios were prepared, and subjected to pressures between 0 and 50,000 atmospheres, at 650° C., for periods between 1 and 6 hr. Fourteen runs were made. After completion of a run, the pressure was rapidly released, while the sample was quenched and then examined by X-ray diffraction.

At pressures below 15,000 atmospheres the phases observed were Ni_2SiO_4 olivine, NiO, and nickel talc. It may be shown that the last two products constitute a metastable assemblage, due to incomplete reaction. Above 20,000 atmospheres, olivine disappeared and in its place a spinel phase appeared, together with NiO and talc. Between 15,000 and 20,000 atmospheres, spinel, olivine, talc and NiO appeared simultaneously. A run at 50,000 atmospheres, using a mix containing 20 per cent excess $Ni(OH)_2$ over that required to form the normal orthosilicate, produced spinel and NiO. Talc was absent.

A sample of Ni_2SiO_4 olivine was prepared by sintering a tablet of intimately mixed NiO and SiO_2 at 1,400° C. for 6 hr. The tablet was then crushed, reformed and resintered, this procedure being repeated twice. A sample thus prepared was subjected to 50,000 atmospheres and 700° C. for 2½ hr. About 80 per cent conversion to the spinel form was observed. Similar runs were carried out at 30,000 and 20,000 atmospheres. A small amount of spinel formed at 20,000 atmospheres, but curiously, no spinel formed at 30,000 atmospheres.

Mixtures of silicic acid, hydrous germanium dioxide, and nickel hydroxide were prepared at intervals in the composition range between Ni_2GeO_4 and Ni_2SiO_4. These were run at 33,000 atmospheres and 650° C. A complete series of spinel solid solutions was found to exist between Ni_2GeO_4 (normally a spinel) and Ni_2SiO_4. The solid solutions obeyed Vegard's law.

It is concluded that the new phase is the spinel modification of Ni_2SiO_4 and that the equilibrium pressure for the olivine–spinel transition in Ni_2SiO_4 at 650° C. is about 18,000 atmospheres. Ni_2SiO_4 spinel is green, transparent, and has a lattice parameter of 8·044 Å. The calculated density is 5·34 gm./c.c., which is about 9 per cent higher than that of the olivine modification.

The experimental transition pressure is in good agreement with the value calculated (54,000 atm.) from zero pressure equilibria at 1,500° C. in the system $Ni_2GeO_4 - Ni_2SiO_4$ if a reasonable allowance is made for the effect of temperature on transition pressure. A detailed description of equilibria in this system, and the calculations based upon it, will be published elsewhere.

Wentorf's failure to synthesize Ni_2SiO_4 spinel at much higher pressures than I used may be due to the different type of apparatus employed[5]. We both used mixtures of $Ni(OH)_2$ and silicic acid as starting materials, and operated at temperatures sufficiently high to cause the components to react and form Ni_2SiO_4. However, the uniaxial device which I used permits rapid quenching—in particular, the water vapour pressure can be instantaneously released. Published descriptions[5] suggest that Wentorf's apparatus may not have been able to quench the reaction products as quickly; furthermore, the quenching may be carried out in the presence of a high pressure of water vapour (from the pyrophyllite pressure medium). Thus it is possible that any spinel formed at high pressures afterwards became inverted to olivine during the quench.

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Potassium–Argon Ages of Some Rocks from the South Atlantic

THE ages of micas separated from a number of rocks from the South Orkney Islands have been determined using the potassium–argon method.

In these particular measurements, the total volume of argon evolved on fusion of the samples was measured by means of a McLeod gauge, and a correction applied for atmospheric contamination using a mass spectrometer to resolve the argon isotopes. Potassium analyses were made with a flame photometer.

All the rocks measured were quartz–mica–schists[1], eight from Signy Island and one from Moe Island nearby (composite sample contains Moe Island material *H*.1365.1). Analytical results and calculated ages are given in Table 1.

Table 1

Mineral	Ref. No.	Weight of sample (gm.)	K_2O (per cent)	Total argon (mm.^{3})	Approximate age (m. yr.)
Biotite	*H*. 60·3	6·6570	4·22	0·177	176
Biotite	*H*. 86·1	12·0716	7·51	0·635	199
Biotite	*H*. 154·8	14·3500	8·38	0·877	183
Biotite	*H*. 164·2	7·3086	4·75	0·226	184
Biotite	*H*. 205·2	8·9206	5·13	0·323	195
Biotite	*H*. 507·3	6·2023	4·00	0·181	195
Muscovite	*H*. 507·3	6·9700	6·38	0·306	176
Biotite	*H*. 1,369·1	6·2587	4·12	0·173	189
Biotite	*H*. 1,365·1 *H*. 1,384·1	7·3328	6·23	0·39	193

$\lambda\beta = 4{\cdot}76 \times 10^{-10}$ yr.$^{-1}$. $\lambda_e = 0{\cdot}585 \times 10^{-10}$ yr.$^{-1}$.

Errors in the results arise from inaccurate measurement of the volume, in the estimation of potassium, or in measuring the argon-40/argon-36 ratio. In these measurements, a 1 per cent error in either the proportion of potassium oxide or the volume of radiogenic argon would result in a 1 per cent error in the age. The errors introduced in correcting for atmospheric contamination may be neglected. The total error of measurement is estimated to be less than $\pm$ 5 per cent.

The similarity between all the ages so far measured suggests that argon has not been released by recent weathering. They indicate a Lower-Jurassic–Upper-Triassic age[2] (Jurassic 135 $\pm$ 5 to 180 $\pm$ 5 m. yr., Triassic 180 $\pm$ 5 to 225 $\pm$ 5 m. yr.) either for the original metamorphism of the schists or for some subsequent metamorphism sufficiently intense to have released their radiogenic argon. The basement schists in the South Orkney Islands are overlain by intensely folded greywackes thought to be of carboniferous age[3], and by slightly folded conglomerates of Mesozoic age[4]. Results from the potassium–argon work are consistent with the hypothesis that radiogenic argon already present was released from the schists during the orogeny which folded the overlying greywackes. No mineralogical evidence of retrograde metamorphism of the schists of Signy Island has been reported.

Thanks are due to the Falkland Islands Dependencies Survey and Dr. R. J. Adie for providing the specimens used in this work, from their collection at the University of Birmingham, and to the Department of Scientific and Industrial Research for financing this work. I am indebted to D. H. Matthews of the Department of Geodesy and Geophysics, Cambridge, for suggesting to me the problem and its geological interpretation.

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Age of the Cambrian System

Recent contributions by Wetherill[1] and Holmes[2] lay stress upon studies of the brannerite (so-called 'absite') from Crocker Well, South Australia, as affording critical evidence on the age of the base of the Cambrian System, but dispute the interpretation of the various results (in the range 520–915 million years) arrived at in dating this mineral. The publication of this discussion prompts me to report why these data from South Australia were ignored, as being irrelevant, in my own notes on the geological time-scale[3]. My conclusions were arrived at in the light of a first-hand knowledge of the Australian deposits, which I visited some years ago, and arose from the following considerations.

(1) Statements that the brannerite occurrences are overlain by Precambrian sediments of the Adelaide System are incorrect. The nearest sediments are six or seven miles away[4]. In these Precambrian strata, pebbles of granite are found which are akin to the adamellite forming the country-rock of the mineral deposits—but this in no way helps to fix the stratigraphical age of the brannerite unless this mineral can be shown to be contemporary with the country-rock.

(2) The samples of brannerite for which isotopic assays are available were not collected specifically for geochronological study with full records of paragenesis, but were presumably supplied out of a stock of concentrate from prospecting operations. It is thus not known whether they were derived from "porous shear zones", as claimed by some authors, or from the tight, breccia-like, stockworks of quartz veins which have formed the main source of the mineral. All the brannerite is amorphous due to metamictization. Consequently there is no geological basis for the several divergent hypotheses, postulating loss of radon due to rock porosity, or retention of radon due to well-defined crystallinity, adduced in argument about the discordant ages.

(3) In addition to brannerite the deposits also contain davidite, the two uraniferous titanates often being intimately intergrown. Greenhalgh and Jeffery[5] have shown that a specimen of this davidite, with an age of 1,600–1,700 m.y., is more than twice as old as the brannerite analysed.

(4) Throughout the whole Olary province, there are a great many occurrences of uranium mineralization (nearly all quite small), and the few that have been analysed isotopically show a wide range of ages. The oldest mineralization is roughly coeval with the great lead deposit of Broken Hill nearby. Both at Crocker Well and Radium Hill, primary uranium minerals bearing at least two widely divergent ages occur in a single ore deposit. In such cases it seems reasonable to attribute the younger datings to re-working, not necessarily as a result of late granitization but probably arising as a simple regeneration *in situ* in response to local tectonic conditions. Comparable regeneration is apparent in the Hercynian pitchblende deposits of Western Europe[6] and in many other uranium fields.

(5) There is no evidence that the latest period of regeneration at Crocker Well is Precambrian in age. Within the province there are many post-Proterozoic mineral veins cutting the strata of the Adelaide System, and the last re-working could be coeval with these lodes. The potassium : argon age of 520

m.y. reported by Greenhalgh and Jeffery[5] relates not to a biotite "hand-picked from the rock", as stated by Wetherill, but to a biotite "hand-picked from the brannerite". The two minerals occur intergrown in nests and may well have been re-constituted contemporaneously.

If these interpretations are correct, the brannerite mineralization is completely lacking in stratigraphical definition and contributes no information about Cambrian chronology. The dating of the base of the Cambrian System at about 600 ± 20 m.y., founded on other evidence, remains unassailed.

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CHEMISTRY

A Molecular Complex of Benzene and Hexafluorobenzene

In the course of experiments using mixtures of benzene and hexafluorobenzene, it was observed that a solid was formed on mixing these two substances although this frequently disappeared before the mixing process was complete. This observation has been followed up by the investigation of the freezing points, obtained from cooling curves, of mixtures of known composition of benzene and hexafluorobenzene.

The freezing-point diagram shows two eutectic points occurring at mole fractions of 0·15 and 0·85 of hexafluorobenzene, and melting at about 0·5° C. and −5·5° C. respectively. The diagram shows a maximum melting point of 23·7° C. corresponding to a mixture containing equimolar quantities of the two substances. Benzene and hexafluorobenzene have similar melting points (5·4° C. and 5·0° C. respectively). This clearly demonstrates the formation of a 1 : 1 molecular complex of the two compounds. Less-complete freezing-point diagrams have been obtained for the systems hexafluorobenzene and fluorobenzene, and pentafluorobenzene and benzene. The first of these shows evidence of the formation of a 1 : 1 complex of hexafluorobenzene and fluorobenzene melting at about −3° C., that is, above the melting point of fluorobenzene but below that of hexafluorobenzene. No solid complex appears to be formed between pentafluorobenzene and benzene. The complexes seem to be similar to that formed as a low-melting white solid on mixing aniline and hexafluorobenzene[1].

These complexes may be regarded as examples of the Lewis base–Lewis acid (electron donor-acceptor) or charge–transfer type, of which those between picric acid and a number of aromatic compounds, and between, for example, benzene and *sym*-trinitrobenzene[2], are familiar examples. Fluorine atoms show a strong negative inductive effect which is reflected by the observation that the ionization potentials of polyfluorobenzenes are higher than that of benzene[3], and by the behaviour of polyfluorobenzenes in chemical reactions. In showing a negative inductive effect, the fluorine atom resembles the nitro group, and it is likely that the conditions in polynitrobenzenes which favour the formation of complexes will also occur in polyfluorobenzenes. The substitution of groups showing a positive inductive effect in the 'basic' partners of the complexes should increase the stability of the complexes with hexafluorobenzene. A complex, melting at 34°, has been prepared from hexafluorobenzene and mesitylene. This complex, unlike that between hexafluorobenzene and benzene, is sufficiently stable to allow its crystallization from ether. A similar complex, melting at 56° C., has been obtained between hexafluorobenzene and 2-methylnaphthalene, and this also is sufficiently stable to allow its crystallization from ether.

Complexes of this type are generally characterized by a charge transfer spectrum. In the expectation that a transfer spectrum such as that observed (in the region 2800–3400 Å.) in the case of the complex between benzene and *sym*-trinitrobenzene would be found, a preliminary investigation has been made of the spectra of neat mixtures of benzene and hexafluorobenzene. No absorption band has been found in the region 2900–4000 Å., where the two liquids do not themselves absorb light. The spectra of mixtures of the two compounds have been measured in the region 2300–3000 Å. using ethanol and hexane as solvents. No absorption bands were observed other than those due to benzene and hexafluorobenzene, but it is probable that the concentrations used were too small to allow the formation of significant concentrations of the complex.

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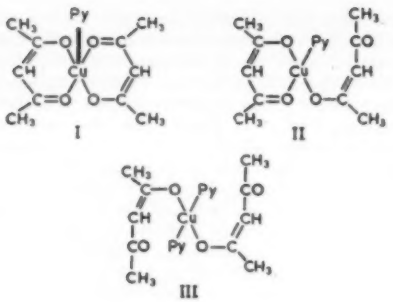
Structure of the 1 : 1-Adducts of Cupric β-Diketone Chelates with Heterocyclic Bases

In a recent communication, Traill[1] raises the question of the structure of the 1 : 1-adducts formed by cupric β-diketone chelates with heterocyclic bases in organic solvents[2]. The same question has been raised privately by others, and it seemed, therefore, that we should seek some further evidence in support of the 5 : co-ordinated structure (I) originally proposed as against the 4 : co-ordinated structure (II) suggested by Traill.

The original attribution of structure I to these adducts was essentially intuitive, and as their low stability makes isolation improbable, a definitive structure determination seems unattainable. We believe, however, that the weight of available evidence strongly favours structure (I).

(1) The stability of acetylacetonates and related compounds is largely due to chelation. If reaction



I II III

with heterocyclic bases may result in the opening of a chelate ring, it is difficult to see why only one ring should be opened, giving (II) rather than both rings giving (III), or even complete displacement giving salts of the type $[Cu(C_5H_5N)_4]^{2+}$ $2(acac)^-$.

(2) Replacement of an oxygen donor by a heterocyclic base normally results in a shift of ligand-field bands to shorter wave-lengths. In this case the shift is very slightly to longer wavelength, an effect paralleled by co-ordination of a fifth ammonia molecule to the $[Cu(NH_3)_4]^{2+}$ ion[3] and in other cases of extension of formally 4 : co-ordinated species to 5 : co-ordination.

(3) Gaussian analysis of the absorption spectra of cupric ethyl-acetoacetate in solvents which do not form adducts shows that the broad absorption band in the visible region consists of two bands with maxima about 550 mμ and 710 mμ ; but when the heterocyclic base adducts are formed, the band at 550 mμ disappears and a new band appears at about 900 mμ. Such drastic changes in the ligand-field absorption would not be explicable if the adduct had structure (II), in which the environment of the copper atom is fundamentally unchanged, but is consistent with the formulation of the adducts as 5 : co-ordinated species[4].

(4) In seeking further evidence for the structure of these adducts, we have now examined the infrared absorption of *M*/40 chloroform solutions of cupric acetylacetonate and cupric ethyl-acetoacetate, and of the same solutions containing *M*/2 γ-picoline (chosen because it gives more complete adduct formation with the minimum concentration of heterocyclic base—under these conditions adduct formation is about 90 per cent complete). In the region 1,500–1,620 cm.$^{-1}$, metal β-diketone chelates show two very strong absorption bands, which are characteristic of the chelate ring system, as they do not appear in keto or enol forms of the free β-diketones or in acetylacetone acetate[5]. Opening of one of the two chelate rings to give (II) should thus be accompanied by a halving of the intensities of these bands. These bands are reported in the acetylacetonates of chromium-(II) and chromium-(III) (ref. 6) at 1,567–9 cm.$^{-1}$ and 1,517–8 cm.$^{-1}$ and of cerium-(III) (ref. 7) at 1,562 and 1,510 cm.$^{-1}$. In our complexes they are observed as follows :

	ν_1	ϵ_1	ν_2	ϵ_2
Cupric acetylacetonate	1,577	380	1,525	530
Cupric acetylacetonate/picoline	1,577	460	1,525	500
Cupric ethyl-acetoacetate	1,597	525	1,522	675
Cupric ethyl-acetoacetate/picoline	1,597	475	1,522	600

where ε is the extinction per mole of β-diketone.

While the accuracy of the extinctions is not high, due to heavy background absorption by the heterocyclic base, it is apparent that they remain almost unchanged, as required for structure (I).

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Catalytic Racemization of *tris*(Ethylenediamine)Cobalt(III) Ion

Although the optical isomers of the *tris*(ethylenediamine)cobalt(III) ion, ($[Coen_3]^{3+}$), are stable indefinitely in aqueous solution at 100° C., complete racemization can be effected at 90° C. in 2 min. in the presence of activated carbon[1]. It has been proposed that racemization is a surface phenomenon, and no decomposition of the complex cation occurs[1]. More recently[2], the isomerization of lævo-*tris*(*l*-propylenediamine)cobalt(III) ion to the equilibrium mixture of the *d* and *l* forms of the complex cation was carried out with activated carbon in aqueous solution at 20° C. in 15 min. These reactions are obviously of the same nature as the rapid equilibration of mixtures of cobalt(III) complexes in various preparative procedures[3,4] brought about by activated carbon, for which no satisfactory explanation has yet been proposed.

We have found, after treatment in aqueous solution with activated carbon, cobalt(III) complexes in general, for example, *tris*(acetylacetone)cobalt(III), *tris*(glycine)cobalt(III), hexammine cobalt(III) ion, *tris*(oxalato)cobaltate(III) ion, invariably contain small amounts of cobalt(II) complexes. The amounts of the latter have been found to depend on the temperature, time of contact with, and the nature of the carbon, as well as the presence or absence of oxygen or air.

In a typical experiment, a solution (10 ml.) containing *d*-*tris*(ethylenediamine)cobalt(III) chloride (0·2 gm.) and activated carbon (B.D.H. gas-absorption) was boiled for 3 min. The optically inactive solution, after removal of the carbon, was made acid with sulphuric acid. The Co^{2+} ion, estimated with triphenylmethylarsonium thiocyanate[5], was found to be 4·2 per cent of the total cobalt. Under the same conditions, but in sulphuric acid (0·1 *N*), the rotation decreased by only 6 per cent, but substantially the same amount of Co^{2+} ion was found (4·0 per cent). Schwarz and Kronig, who first used charcoal as a catalyst in cobalt(III) complex equilibria, failed to detect any cobalt(II) complex or salt, and subsequent workers have overlooked the reducing properties of charcoal.

These observations suggest electron-transfer racemization proceeding through the labile $[Coen_3]^{3+}$ ion, which is decomposed by acid. The carbon appears to serve three functions : (*a*) as a catalyst for the conversion of a small amount of the spin-paired $[Coen_3]^{3+}$ ion to the activated spin-free paramagnetic state which it then (*b*) reduces to the spin-free $[Coen_3]^{2+}$

ion, and (*c*) as a catalyst for the electron transfer process:

$$d[Coen_3]^{3+} \text{ spin-paired} + l[Coen_3]^{2+} \text{ spin-free} \rightarrow l[Coen_3]^{3+} + d[Coen_3]^{2+} \rightleftharpoons l[Coen_3]^{2+}$$

Activated carbon is known to be capable of effecting mild reductions in aqueous solution, for example, ferric to ferrous ion, but would not be expected to reduce the spin-paired $[Coen_3]^{3+}$ ion because of the negative potential of couple $[Coen_3]^{3+}$ spin-paired/$[Coen_3]^{2+}$ spin-free ($E_0 = -0{\cdot}232$ V.)[3]. The potential of the couple $[Coen_3]^{3+}$ spin-free/$[Coen_3]^{2+}$ spin-free, in which both oxidation states are electronically equivalent, would be expected to be very much more positive.

Because of electronic non-equivalence, electron transfer between cobalt(II) and cobalt(III) complexes in aqueous solution is usually slow[6]. For example, the complete electron-transfer racemization of $d[Coen_3]Cl_3$ (0·0551 *M*) in the presence of *d,l*-$[Coen_3]SO_4$ (0·02 *M*) and ethylenediamine (0·24 *M*) in a nitrogen atmosphere at 25° C. required 8 days[7]. Activated carbon (2 gm. in 100 ml. of solution) catalysed the above reaction strongly, and all activity was lost in 2 min. at 25° C.

Racemization was also catalysed by freshly prepared platinum, palladium and rhodium black, and small amounts of cobalt(II) complex could be detected. In the presence of these metals a reducing agent was necessary. This could be adsorbed hydrogen, sulphite ion or even alcohol.

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'Eddy' Diffusion in Chromatography

Under the above title, J. Calvin Giddings[1] has discussed the axial spreading of the solute in chromatographic columns which is responsible for the smearing of zones and in particular the contribution made to this by 'eddy' diffusion.

In 'eddy' diffusion the spreading of the solute is less than could be derived from random walk considerations applied to the carrier fluid, since the uneven velocity distribution causes radial concentration gradients, which by radial molecular diffusion tend to keep the solute closer together in the axial direction.

For turbulent flow in unpacked tubes the appropriate theory has been worked out by Taylor[2]. The velocity distribution in laminar flow also produces a spreading effect which is counteracted by radial diffusion. This case has also been examined by Taylor[3]. A generalized treatment for tubes of arbitrary cross-section and for arbitrary velocity profiles is due to Aris[4].

In our publication[5] on the statistical treatment of mechanisms causing axial spreading we have expressed the view (*loc. cit.*, p. 267) that the velocity distribution (in this case a parabolic one) and the radial molecular diffusion cannot be considered as being statistically independent, the reason being that the second process reduces the effect of the first. Giddings (*loc. cit.*) states that the two processes are independent.

For independent processes, when variances must be added, as we have explained, both the apparent diffusion coefficient D and the height H corresponding to an ideal stage have conserved the property of additivity, since they are proportional to the variance.

However, in order to obtain a reduction in D (or H), Giddings has introduced the additivity in a factor n occurring in the denominator in his equation (1) on the strength of a statement that n is the sum of numbers of steps for the two supposedly independent processes. We fail to see the statistical justification for this procedure.

The fact that, for proper a and b, the form:

$$1/\left(\frac{1}{a}+\frac{1}{b}\right)$$

has the correct limits for $b \rightarrow \infty$ and $a \rightarrow \infty$ is of course insufficient to justify the treatment.

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Variances add[1], for certain independent processes which, by virtue of an elementary random event, contribute directly to the variance. Lateral diffusion is not such a process. The variance is caused by non-equilibrium established by the difference in downstream velocity from point to point. Lateral diffusion acts to reduce this variance as can be seen by the inverse relationship between variance or plate height and diffusion coefficient. The random change in velocity which occurs along a given stream path, as found in packed columns but not in capillary columns, also reduces the variance. Both factors act independently to exchange molecules between fast- and slow-moving regions.

The fact that independent processes do not necessarily contribute additive terms to the variance can be seen by use of a simple example which, unlike the above, can be accounted for in an exact fashion. Let the transition between two discrete species, A_1 and A_2, with downstream velocities v_1 and v_2 occur by two independent mechanisms. The forward and reverse rate constant for the first mechanism are k_1 and k_2, respectively, and for the second mechanism, k_1' and k_2'.

Using a method developed elsewhere[2] for obtaining asymptotic expressions for plate height with complex kinetics, we obtain:

$$H = \frac{2R\,(1-R)\,(v_1-v_2)}{k_2 + k_2'}$$

where R, the ratio of average solute to average fluid velocity, is an equilibrium property. Rate effects appear only in the denominator, and, in fact, are additive in the denominator as proposed for eddy diffusion[3]. We see that "the second process reduces the effect of the first"[1], but the processes are, none

the less, physically independent. This fact in no way depends upon having a single-step mechanism. If solute molecules are shuttled laterally by two independent diffusion processes, it can be shown[1], as expected, that the diffusion coefficients add in the denominator, one reducing the effect of the other. In close analogy with eddy diffusion, both diffusion processes "act independently to exchange molecules between fast- and slow-moving regions". It is, in fact, possible to assign an effective coefficient of lateral diffusion (even though lateral diffusion, as such, is not necessarily involved) to describe the effect of velocity variations along a streamline. A close analysis of this shows that the effective diffusion coefficient varies with the distance across the interstice while the molecular diffusion coefficient is constant[4]. At any point these two are additive in the denominator, but the addition of the averages of the two, and this is essentially what was done in the earlier treatment[3], is an approximation. I am at present looking for an improvement of the admittedly approximate derivation previously given.

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BIOCHEMISTRY

Exclusion of Inulin from Solutions of Hyaluronic Acid

The partition of inulin between buffer and solutions containing hyaluronic acid has been observed by dialysing inulin to equilibrium between the two solutions. 'Millipore *HA*' membranes were used which are readily permeable to inulin but impermeable to hyaluronic acid. The hyaluronic acid was prepared from ox synovial fluid by the method of Ogston and Stanier[1]; inulin was Kerfoot biochemical reagent twice 'recrystallized' from dilute ammonia.

The partition depended on the concentration of hyaluronic acid according to $\log K = 2w$, where K is the ratio of concentration of inulin in buffer to that in the hyaluronic acid solution at equilibrium and w is the weight of hyaluronic acid/100 ml. The partition was independent of the concentration of inulin, the direction of equilibration, *p*H and ionic strength. Thus at $w = 0{\cdot}2$, $K = 2{\cdot}5$, and this can be thought of as the exclusion of inulin from 60 per cent of the volume of the hyaluronic acid solution. These results have a bearing on the use of inulin for determining the volumes of tissue spaces.

The effect is not peculiar to inulin, but is shown by other carbohydrates and by proteins, to degrees depending on their effective molecular volumes; this suggests that the mechanism of exclusion is steric.

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Alkaline C-1 Fructose-1,6-diphosphatase: Evidence for its Participation in Photosynthesis

Pathways proposed for the conversion of carbon dioxide to hexose monophosphate during photosynthesis require the cleavage of the phosphate linked to carbon-1 of either fructose-1,6-diphosphate, or sedoheptulose-1,7-diphosphate, or both[1–3]. A C-1 alkaline phosphatase specific for fructose-1,6-diphosphate has been prepared from spinach leaves and characterized by Racker and Schroeder[3]. This enzyme was not thought to participate in photosynthesis since it did not appear to be localized in the chloroplasts. Recently it was reported[4] that pea chloroplasts isolated in various aqueous media lost about 90 per cent of their ribulose-1,5-diphosphate carboxylase, an enzyme considered to play an essential part in photosynthesis[1]. Since this finding indicated that photosynthetic enzymes were not necessarily retained in chloroplasts isolated by the usual procedures, the possible function and intracellular distribution of alkaline fructose-1,6-diphosphatase in photosynthetic tissues were re-investigated.

A survey of a number of plants and bacteria disclosed that alkaline fructose-1,6-diphosphatase was widely distributed in photosynthetic tissues. In addition to spinach[3] and pea[5] leaves, alkaline fructose-1,6-diphosphatase has been demonstrated in cell-free extracts of photosynthetic tissues from barley, alfalfa, *Spirodela*, the fern *Pteris gautheri*, the algae *Euglena gracilis* and *Chlamydomonas reinhardii*, and the photosynthetic bacteria *Rhodospirillum rubrum* and *Chromatium*. Conversely, the enzyme was not found in extracts of pea root or an apoplastidic strain (streptomycin-bleached) of *Euglena gracilis*. The activity of the enzyme in etiolated pea leaves was less than 5 per cent of the maximum activity found in green pea leaves. In related work in which quantitative measurements were made of the levels of various enzymes in growing pea leaves, it was observed that the alkaline fructose-1,6-diphosphatase activity of the leaf followed a pattern of development similar to that previously ascertained for a number of photosynthetic enzymes[6]. The above evidence attests to a close correspondence between alkaline fructose-1,6-diphosphatase and chloroplast development and function.

Intracellular distribution studies of cell particulates isolated in either 0·5 *M* sucrose or 0·35 *M* sodium chloride were inconclusive because of the apparent loss of most of the photosynthetic enzymes from the chloroplasts during the fractionation procedure. However, this work did show that a small amount of

Table 1. The Distribution of Alkaline Fructose-1,6-Diphosphatase in Pea Leaf and *Euglena* Fractions Isolated in Non-aqueous Media

Plant material	Density of isolated fraction	Fructose-1,6-diphosphatase*	
		Per cent activity in fractions	Activity per chlorophyll (μmoles P/min./mgm. chlorophyll)
Pea leaf	<1·34	68	0·52
	1·34–1·40	28	0·57
	>1·40	4	0·55
Autotrophic *Euglena*	<1·29	88	0·32
	1·29–1·36	9	0·57
	>1·36	3	0·57

* The reaction mixture contained *tris*(hydroxymethyl)aminomethane buffer, *p*H 8·5 (100 μmoles/ml.), fructose-1,6-diphosphate (3·5 μmoles/ml.), magnesium chloride (5 μmoles/ml.), and ethylenediamine tetraacetate (1·6 μmoles).

the total cellular alkaline fructose-1,6-diphosphatase was retained in the chloroplasts to an extent equivalent to that reported previously for ribulose-1,5-diphosphate carboxylase[4]. Furthermore, the specific activity of the enzyme in the chloroplast fraction was higher than in the other fractions. More informative results were obtained from experiments in which fractionations of pea leaves and *Euglena* cells were carried out in non-aqueous media. The procedure detailed by Stocking[7] for spinach leaves was followed, except that the *Euglena* cells were broken by grinding with glass beads (0·1–0·25 mm. diameter) with a pestle and mortar. This fractionation procedure produced an enrichment of chloroplast material in the low-density fractions[4,7]. The results in Table 1 show that the majority of the alkaline fructose-1,6-diphosphatase activity was associated with the lightest fractions and that the activities in the various fractions closely followed the distribution of chlorophyll. The low value for the activity of fructose-1,6-diphosphatase expressed on a chlorophyll basis in the *Euglena* fraction less dense than 1·29 was accounted for by the presence in this fraction of unbroken *Euglena* cells. Separate experiments showed that no significant amounts of protein were released from unbroken cells during the fractionation procedure. These distribution studies suggest that the alkaline fructose-1,6-diphosphatase of pea leaf and *Euglena* is localized in the chloroplasts.

Although it has been shown that leaf extracts also contain an acid fructose-1,6-diphosphatase[3], no evidence has been obtained to implicate this enzyme in photosynthesis. The developmental studies and intracellular distribution experiments indicated that the leaf acid fructose-1,6-diphosphatase did not participate directly in photosynthesis, nor was it localized in chloroplasts. Extracts of *Chromatium*, an obligate anaerobe and phototroph, contained only the alkaline fructose-1,6-diphosphatase.

On the basis of the observations mentioned above, it is suggested that alkaline fructose-1,6-diphosphatase constitutes an integral part of the enzymic mechanism for the photosynthetic conversion of carbon dioxide to carbohydrates.

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Transiodination of Proteins during Enzymic De-iodination of Thyroxine

DURING the enzymic de-iodination of thyroxine labelled with iodine-131, triiodothyronine and related compounds with tissue homogenates, a fraction of the radioactivity consistently appeared in a material of $R_F = 0$ in various chromatographic systems[1]. The amount of 'origin' radioactive material found on chromatograms increased almost in parallel with the de-iodination of the substrate. With the identification of ferrous ions and flavin as activators for thyroxine dehalogenase[2], the formation of this material was found to be dependent only on flavin derivatives. The nature of the 'origin' iodine-131 labelled material was studied with purified preparations of thyroxine dehalogenase[3]; it was found to be radioiodinated protein(s) present in the tissue enzymic extracts, as described below.

L-Thyroxine labelled with iodine-131 was incubated at 37° with thyroxine dehalogenase prepared from rabbit skeletal muscle, at *p*H 7·1 (ref. 3). The radioactive products of the reaction were analysed by paper chromatography and electrophoresis. Full experimental details have been described elsewhere[3]. Next to iodide, the material of $R_F = 0$ was the only other major labelled product formed. As shown in Fig. 1, the rate of formation of iodide was only slightly diminished in the absence of flavin mononucleotide in a ferrous ion-activated enzyme system, whereas the formation of radioactive 'origin' material was almost abolished.

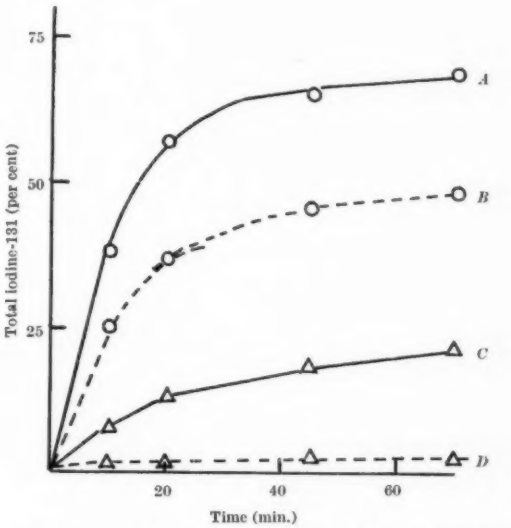

Fig. 1. Formation of radioactive iodide (○) and 'origin' material (△) as the principal products of enzymic de-iodination of thyroxine labelled with iodine-131 by rabbit skeletal muscle thyroxine dehalogenase as measured by chromatographic analysis. Each flask contained in 2·5 ml. *tris*-hydrochloric acid – potassium chloride buffer[3], *p*H 7·1, 5·0 mμmoles of L-thyroxine, 12 mgm. of enzyme of × 72 purification. Additions: *A* and *C*, 0·035 μmole ferrous ion and 0·1 μmole flavin mononucleotide; *B* and *D*, 0·035 μmole ferrous ion alone

After incubation of labelled thyroxine with a fully activated enzyme, the 'origin' material was separated by paper chromatography. It was non-dialysable and precipitated with trichloracetic acid; it was then digested with crude trypsin or pancreatin. The hydrolysate on chromatographic analysis was found to contain most of the iodine-131 as 3-iodo-tyrosine with traces of 3 : 5-diiodotyrosine, which suggested the presence of iodinated proteins.

In order to confirm that proteins were iodinated with the iodine released from thyroxine, extraneous proteins were added in relatively large amounts to the muscle enzyme extract. Insulin and egg-white lysozyme were most suitable because of the ease of distinguishing from muscle proteins by electrophoresis and their feeble thyroxine-binding activity (thyroxine

Table 1. Iodination of Insulin and Egg-White Lysozyme present during Incubation of ^{131}I-labelled L-Thyroxine (T_4) with Rabbit Skeletal Muscle Thyroxine Dehalogenase

Protein added	Amount (mgm./ml.)	T_4 de-iodinated in 60 min. (per cent)	Percentage of ^{131}I protein-bound	Percentage of protein-bound ^{131}I recovered in added protein
None	—	83·6	15·1	—
Insulin	2·0	72·7	22·0	50·2
	5·0	60·0	28·7	68·5
Lysozyme	2·0	74·4	18·2	31·8
	5·0	70·1	24·5	43·6

Conditions identical to those in Fig. 1. Enzyme fully activated with ferrous ions and flavin mononucleotide.

dehalogenase can only attack 'free' substrate). Both these proteins were readily radioiodinated in the presence of radioactive thyroxine and the enzyme (Table 1), and on tryptic hydrolysis after separation by paper electrophoresis gave rise to labelled monoiodotyrosine. Since free iodotyrosines were not a product of action of dehalogenase, the transfer of iodine atoms from thyroxine to the tyrosyl radicals of added proteins represents a true process of transiodination.

Observations reported elsewhere[3] have suggested that the iodination of proteins is not a secondary process of a flavin-mediated oxidation of the iodide released by the dehalogenase but rather a direct substitution. It was proposed that the contribution of the flavin was to release part of the iodine atoms at a level of oxidation necessary for iodination under conditions of incubation. It should be noted that the fraction of thyroxine iodine available for tyrosyl substitution was twice the fraction of protein-bound iodine-131 shown in Fig. 1 or Table 1 according to $R.H. + 2I^{*} \rightarrow R.I. + R.I.$

Since enzymic dehalogenation of thyroxine is not a peroxidative process[3], the iodination of proteins reported here does not resemble the 'transiodination' of some iodotyrosines and iodothyronines with peroxidase[4].

The physiological significance of transiodination of proteins coupled to a de-iodination of thyroid hormones, which had hitherto remained unnoticed, remains doubtful. Considering the extremely high ratio of proteins to thyroxine in tissues, it would be difficult to demonstrate such a transiodination *in vivo*.

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Stabilization by Manganous Ions of Ribosomes from Embryonic Plant Tissue

Preparations of microsomal ribonucleoprotein (ribosomes[1]) from sources such as yeast, pea shoots, rat liver and clover leaves have been shown to consist largely of particles with sedimentation coefficient approximately 70 *S* (refs. 2–5). These particles are stable when dialysed against solutions containing magnesium ions at concentrations between 1 and 2 m*M*; but removal of magnesium by dialysis or addition of chelating agents leads to dissociation of the 70 *S* particles with production of sub-units of sedimentation coefficients 50 *S* and 30 *S*. By contrast, I have found with ribosomes from wheat germ that in order to preserve intact 70 *S* particles on dialysis, manganese and calcium are required in addition to magnesium in the dialysing solution.

Wheat germ was blended for 2 min. with four times its weight of a solution containing 5 m*M* magnesium chloride, 2 m*M* calcium chloride, and 0·025 *M* *tris*(hydroxymethyl)aminomethane/hydrochloric acid (*tris*) buffer at *p*H 7·3. The ribosomes were isolated by two stages of fractional centrifugation in the same buffered medium[3]. The material thus obtained was homogeneous on examination in the analytical ultracentrifuge, and contained only the 70 *S* component (Fig. 1*a*).

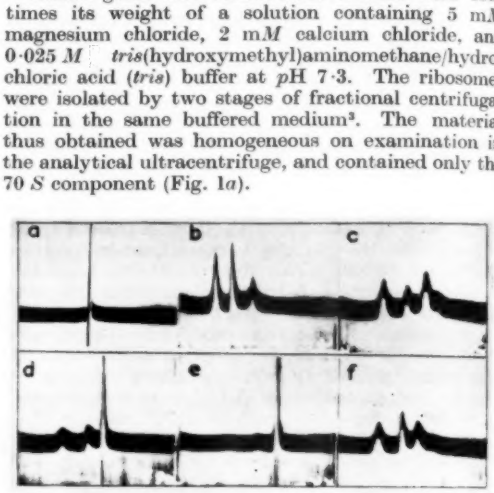

Fig. 1. Ultracentrifuge diagrams of wheat germ ribosomes: *a*, as prepared in 0·025 *M* *tris*, 5 m*M* magnesium sulphate, 2 m*M* calcium chloride; *b*, dialysed v. 0·025 *M* *tris*, 1 m*M* magnesium sulphate; *c*, dialysed v. 0·025 *M* *tris*, 2 m*M* magnesium sulphate; *d*, dialysed v. 0·025 *M* *tris*, 1 m*M* magnesium sulphate, 0·2 m*M* manganous chloride; *e*, dialysed v. 0·025 *M* *tris*, 1 m*M* magnesium sulphate, 0·2 m*M* manganous chloride, 0·5 m*M* calcium chloride; *f*, dialysed v. 0·025 *M* *tris*, 1 m*M* magnesium sulphate, 0·2 m*M* nickel sulphate. All solutions at *p*H 7·3. Ribosome concentration: *a*, 10 mgm./ml., *b–f*, 5 mgm./ml. All photographs taken after 9 min. at 42,000 r.p.m. Sedimentation left to right

In order to test the stability of these ribosomes under different conditions, 1·0-ml. samples at a concentration of about 5 mgm./ml. were dialysed against three changes of 300 ml. of the test solution for 24-hr. periods. The dialysed sample was then examined in the ultracentrifuge.

It will be seen from Fig. 1 that dialysis against a buffer containing 1·0 m*M* magnesium sulphate led to almost complete dissociation of the 70 *S* particles, and increasing the concentration to 2·0 m*M* was only partially effective in counteracting this dissociation. Dialysis against higher concentrations of magnesium led to production of aggregated material. When 0·2 m*M* manganous chloride was added to the 1·0 m*M* magnesium, however, the dissociation was largely suppressed. Increasing the manganese concentration to 0·5 m*M* further repressed the dissociation, but the most effective medium for preserving the 70 *S* particles intact on dialysis has been found to be a *tris* buffer containing 1·0 m*M* magnesium sulphate, 0·2 m*M* manganous chloride and 0·5 m*M* calcium chloride (Fig. 1*e*). In the presence of manganese, the effect of calcium was specific. Increasing the magnesium concentration did not have the same effect. In the absence of magnesium or calcium, complete dissociation of the 70 *S* particles was not prevented by addition of manganese to 0·5 m*M* concentration.

Similar concentrations of divalent salts of nickel, cobalt, iron, zinc and copper were all closely similar, and all much less effective than manganese in stabilizing the 70 *S* particles (Fig. 1*f*).

We have also observed a similar stabilizing effect of manganous ions on ribosomes prepared from pea seeds.

Analysis of wheat germ ribosomes for manganese[6] gave values of 0·12–0·14 μgm. per mgm. ribonucleic acid. The extract from which they were isolated contained approximately ten times as much manganese per mgm. ribonucleic acid.

This dissociation of 70 *S* particles on dialysis in the presence of magnesium is in contrast to the reported behaviour of ribosomes prepared from the sources referred to earlier[2–5]. A further difference exhibited by wheat germ ribosomes is the irreversibility of the dissociation which occurs on dialysis either in the presence or absence of magnesium. Further dialysis of the dissociated particles against the buffer containing magnesium, manganese and calcium, which was effective in repressing dissociation, led to aggregation of the 50 *S* and 30 *S* components, but little if any 70 *S* component was regenerated.

It is possible that the difference in properties of the ribosomes studied here may be due to their having originated from embryonic rather than fully differentiated tissue. Further work will be required to check this.

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The β-D-Fucosidase Activity of Mammalian Tissues and its Relation to other Glycosidases

An earlier communication[1] described the occurrence of the enzyme α-L-fucosidase in mammalian tissues. These tissues have now been surveyed for β-D- and β-L-fucosidase activity, using the appropriate *p*-nitrophenyl fucosides as substrates. The β-D-fucoside was hydrolysed by all the mammalian tissues that were studied, and also by an extract from the limpet, *Patella vulgata*, but no β-L-fucosidase activity was detected anywhere.

Table 1 gives figures for some of the richest sources of β-D-fucosidase, alongside comparable figures for β-D-galactosidase[2]. Since fucose is 6-deoxygalactose, β-D-fucosidase could be identical with β-D-galatosidase, but the parallelism which would then be expected between the two enzyme activities, within at least a single species, was not observed. Inhibition experiments with the appropriate aldonolactones[3] are shown in Table 2. While the results for the preparations from the rat are consistent with the action of a single enzyme, with a higher affinity for the galactose than for the fucose residue, the figures for ox liver suggest the very opposite. In any event, the enzyme or enzymes in the two species are evidently quite different. This is reflected in differences I have observed in the *p*H-activity curves.

Whether or not it is due to β-D-galactosidase, the β-D-fucosidase activity of mammalian tissues is considerable. D-fucose has not been reported in animals, but both D- and L-fucose occur in plants. Although L-fucose has been positively identified in mammalian preparations in at least two instances, in other cases its presence has been inferred from evidence that does not distinguish it from the D-enantiomorph. As with other galactose derivatives, the biological interconversion of D- and L-fucose by an end-to-end oxidation-reduction would appear to be feasible.

Table 1. β-D-Fucosidase and β-D-Galactosidase Activities of Mammalian Tissues

	β-D-Fucosidase			β-D-Galactosidase		
	Pig	Ox	Rat	Pig	Ox	Rat
Liver	22,800	29,700	1,910	14,500	7,260	4,710
Kidney	31,700	1,010	4,050	28,550	1,860	11,400
Epididymis	360	900	3,300	970	1,740	47,300

Results expressed as μgm. *p*-nitrophenol (fucosidase) or *o*-nitrophenol (galactosidase) liberated per gm. moist tissue in 1 hr. at 37°.

Table 2. Inhibition of β-D-Fucosidase and β-D-Galactosidase by D-Aldonolactones

Source of enzyme	β-D-Fucosidase		β-D-Galactosidase	
	Fucono-lactone	Galactono-lactone	Fucono-lactone	Galactono-lactone
Rat epididymis	0	56	0	60
Rat liver	0	64	0	65
Ox liver	0	0	32	0

The experiments were done at *p*H 4 in 0·05 *M* acetate buffer with 1 m*M* substrate and inhibitor. Results are expressed as inhibition per cent.

One interesting observation made in this work was a great variation in the β-D-fucosidase activity of cow ovary, which was found to be related to the amount of luteal tissue present. The corpus luteum had a mean activity of 1,000 units per gm., whereas the remainder of the tissue had only one-tenth of this activity and the enzyme was virtually absent from the follicular fluid. This phenomenon was shown to extend to α-L-fucosidase, β-D-galactosidase, α-D-mannosidase, β-D-glucuronidase and β-*N*-acetyl-D-glucosaminidase in cow ovary. While the physiological significance of these results is obscure, they point to a common function for all six enzymes, and to direct or indirect control of their activity by the anterior pituitary. As already shown for the other four enzymes[4], β-D- and α-L-fucosidase in epididymis and uterus respond to castration by steep falls in activity.

Grateful acknowledgment is made to Dr. J. Conchie for help with some of the enzyme assays, and to Mr. A. McAllan for skilled technical assistance.

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Biosynthesis of Squalene by the Annelid *Lumbricus terrestris*

Large amounts of sterols have been shown to be present in many of the invertebrates, although cholesterol does not seem to occur predominantly among the more primitive animals. Instead, as Bergmann has pointed out, the lower phyla especially appear to contain a diverse group of sterols with structures which are more similar to those of plants[1]. Moreover, since some Protozoa and insects have been shown to require dietary cholesterol[2], it has been suggested that the invertebrates cannot synthesize their own sterols, but rather modify the steroid nucleus present in their food sources.

Mevalonic acid, isolated as a growth factor for a strain of *Lactobacillus acidophilus*[3], has been shown to be a precursor of squalene and cholesterol with *in vivo* experiments using the rat[4], and in isolated mammalian[5,6] and yeast systems[7]. The extent of mevalonic acid utilization by invertebrate animals is now being studied to determine by isolation and identification of radioactive components of the non-saponifiable fraction what genetic blocks exist in various phyla.

A species from the phylum Annelida has been found to convert mevalonic acid to squalene, but is incapable of cyclizing the hydrocarbon. Specimens of *Lumbricus terrestris* were placed in moist cellulose powder which contained 0·1 per cent 'Moldex' and 10,000 units penicillin (Lilly) and were allowed to burrow in this medium for six days to remove symbionts undoubtedly present in the alimentary canal. Additional penicillin solutions were added on the second and fourth days, and the worms averaging 6–8 in. long and 3–4 gm. in weight remained quite lively. Potato agar plates streaked with cœlomic fluid from a treated worm showed only a few small, slow-growing colonies of *Clostridium* sp. The mevalonic acid solution contained penicillin and aureomycin and was always injected into the cœlomic cavity.

In one experiment, 11 animals were administered 6 μm. 2-^{14}C mevalonic acid (Tracerlab) with a total activity of 700,000 counts/min. Twenty-four hr. later they were homogenized in a Waring blendor with water in a nitrogen atmosphere prior to four extractions at *p*H 11 with double volumes of petroleum ether (30–60° C.)/ethanol (9 : 1 mixture by vol.). The sodium sulphate-dried extracts were combined for solvent removal *in vacuo*, and the residue with approximately 10 per cent of the injected activity was dissolved in light petroleum ether for chromatography on an acid-washed alumina (Merck) column. After 150 ml. petroleum ether wash had passed through the column, the squalene was eluted with a 100 ml. mixture of benzene/petroleum ether (1 : 9). Solvents were removed *in vacuo* and the residue was counted on a Nuclear Chicago gas-flow counter. Eighteen mgm. squalene (Eastman Organic Chemicals), previously redistilled and purified by alumina chromatography, were added as carrier to the colourless oil, which weighed 7 mgm. and had an activity of 16,000 counts/min. The hexahydrochloride derivative, prepared according to the Heilbron method modified by Loud and Bucher[4], had a specific activity of 475 c.p.m./mgm., while a second recrystallization from acetone resulted in an increase to 630 c.p.m./mgm. Results of an elemental analysis of the squalene hexahydrochloride synthesized by this micro procedure corresponded to the theoretical percentages of carbon (57·26), hydrogen (8·92), and chlorine (33·83) (analysis by Geller Lab. carbon (57·21), hydrogen (8·85), chlorine (34·10)).

In another experiment purified carrier squalene was added to the non-saponifiable fraction obtained from another group of animals prior to separation by alumina chromatography. The squalene fraction had a specific activity of 86 c.p.m./mgm. before the derivative was made, and the activity remained relatively constant over four recrystallizations (Table 1).

Table 1. Recrystallization of ^{14}C Squalene Hexahydrochloride to Constant Specific Activity

Recrystallization	Weight (mgm.)	Counts/min.	C.p.m./mgm. squalene hexahydrochloride (Corrected to infinite thinness)
1	9·7	1,320	185
2	7·6	1,112	182
3	5·6	840	176
4	5·0	767	176

The alumina column was developed further with 1 per cent acetone in petroleum ether and radioactivity was found associated with a β-carotene band. β-carotene (N.B.C. recrystallized) was added as carrier, but after crystallization it was inactive. Then several milligrams of coenzyme Q_{10} (Merck) were dissolved in an ether solution of the labelled compound and chromatographed on 'Florisil'. Though the activity moved with the carrier, on recrystallization coenzyme Q_{10} was inactive and the bulk of the carbon-14 compound was recovered in the supernatant liquid. Rechromatography on alumina with very small cuts assayed indicated that the radioactive compound moved slightly faster than the coloured component, but its identity is unknown.

Cholesterol was eluted from the alumina column with acetone/petroleum ether (1 : 1) and after removal of solvent was precipitated with 1 per cent digitonin in 80 per cent ethanol. The resulting voluminous complex was inactive. In addition, the homogenate of three animals injected with labelled mevalonic acid was subjected to saponification procedures which involved refluxing on a steam-bath for 8 hr. with 2 *N* alcoholic potassium hydroxide. Five petroleum ether extractions were made, combined, dried over sodium sulphate, and the activity measured after removal of solvent *in vacuo*. This residue with 6,200 counts/min. was dissolved in 6 ml. ethanol and 3 ml. of 1 per cent digitonin solution was added. After ethanol and ether washes 77·2 mgm. of digitonide were plated and found to be devoid of radioactivity.

Thus, the conversion of mevalonic acid to squalene has been shown to occur in the phylum Annelida. However, a genetic block on the pathway from squalene to the β-hydroxy and/or 5 αH sterols appears to exist in the species studied.

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Phosphate Esters produced by Mammalian Skin from 2-Deoxy-D-glucose

In a recent report concerning the phosphate esters synthesized by skin maintained on various substrates *in vitro*[1] we described the formation of two phosphate esters from 2-deoxy-D-glucose which were not detected with skin maintained on D-glucose. One of these was identified as 2-deoxy-D-glucose-6-phosphate; this communication describes the identification of the second ester.

Three phosphate esters commonly formed from D-glucose-6-phosphate in living cells are D-fructose-6-phosphate, D-glucose-1-phosphate and D-gluconate-6-phosphate. 2-Deoxy-D-glucose-6-phosphate cannot, for obvious structural reasons, be converted to a product corresponding to D-fructose-6-phosphate, so it seemed likely that the unknown ester was either 2-deoxy-D-glucose-1-phosphate or 2-deoxy-D-gluconate-6-phosphate. The latter possibility was chosen for investigation since 2-deoxy-D-glucose-1-phosphate is probably unstable.

Oxidation of D-glucose-6-phosphate with bromine is known to yield D-gluconate-6-phosphate[2] so that a similar oxidation of 2-deoxy-D-glucose-6-phosphate appeared to be a suitable method for preparing 2-deoxy-D-gluconate-6-phosphate. Barium 2-deoxy-D-glucose-6-phosphate was obtained as described previously[1] by the phosphorylation of 2-deoxy-D-glucose using yeast hexokinase and adenosine triphosphate. The oxidation was carried out with bromine in aqueous solution at 40° C. for 22 hr. in the presence of sodium benzoate as a buffer. After cooling, the precipitated benzoic acid was removed by filtration, the solution aerated until colourless and treated with silver carbonate to remove bromide ions. The resultant solution was passed through an 'Amberlite *IR*-120' (H^+ form) column, concentrated under reduced pressure and extracted with chloroform to remove the remaining benzoic acid. It was then neutralized with calcium carbonate and the filtered, neutral solution freeze-dried to a white powder.

A solution of the product, converted to the sodium salt by treatment with sodium carbonate, was mixed with an extract prepared from skin slices (about 20 mgm.) previously incubated for 24 hr. at 37° C. on a medium consisting of 0·02 *M* 2-deoxy-D-glucose in Krebs–Ringer phosphate labelled with 15 μc. phosphorus-32 per ml. This mixture was subjected to two-dimensional paper chromatography and a radioautograph of the chromatogram prepared; the experimental procedure was exactly as previously described[1] with other reference compounds. The spot obtained on spraying the paper chromatogram for phosphate esters coincided exactly with the position of the unidentified spot produced on the radioautograph; the same result was obtained on repetition of the experiment. The R_I values obtained in propan-1-ol/aq. ammonia solution (sp. gr. 0·880)/water (60:30:10 by vol.) were 0·95 and 1·03; in picric acid/2-methyl propan-2-ol/water (4:8:20, w/v/v) they were 0·78 and 0·78. Therefore the product of bromine oxidation of 2-deoxy-D-glucose-6-phosphate appears to be identical to the previously unidentified phosphate ester produced by skin in the presence of 2-deoxy-D-glucose.

However, chemical investigation of the synthetic product showed that it was not calcium 2-deoxy-D-gluconate-6-phosphate. Treatment with *N*-hydrochloric acid at 100° C. for 30 min. produced no hydrolysis of the phosphate group whereas the phosphate group in 2-deoxy-D-glucose-6-phosphate is 70 per cent hydrolysed under these conditions[3]; it is unlikely that the formation of a carboxylic acid group at C(1) would decrease the acid-lability to this extent. The periodate consumption, measured spectrophotometrically[4], was 1 mole per 111 gm. compared to the calculated consumption of 1 mole per 158 gm. These properties and the elementary composition are consistent with a calcium hexonate-6-phosphate (found: C, 22·0; H, 3·7; P, 9·3; $C_6H_{10}O_{10}PCa_{1\frac{1}{2}}$ requires C, 21·6; H, 3·0; P, 9·3 per cent). Oxidation of 2-deoxy-D-glucose-6-phosphate at C(2) as well as at C(1) could lead to D-gluconate-6-phosphate or D-mannonate-6-phosphate. Comparison of the products of bromine oxidation of both D-glucose-6-phosphate and of 2-deoxy-D-glucose-6-phosphate gave indistinguishable results for periodate consumption and infra-red spectra, but on two-dimensional chromatograms different R_I values were obtained. A pure sample of D-mannonate-6-phosphate could not be obtained from D-mannose-6-phosphate; even after four days oxidation some D-mannose-6-phosphate remained. Chromatography of the reaction mixture showed a component having R_I values consistent with those obtained for the oxidation product of 2-deoxy-D-glucose-6-phosphate. As the bromine oxidation product of 2-deoxy-D-glucose-6-phosphate is indistinguishable from calcium D-gluconate-6-phosphate by periodate consumption, infra-red absorption, acid hydrolysis and elementary composition but differs chromatographically; this product is almost certainly calcium D-mannonate-6-phosphate.

Since the previously unidentified phosphate ester produced by skin from 2-deoxy-D-glucose is identical chromatographically with the product of bromine oxidation of 2-deoxy-D-glucose-6-phosphate this ester is presumably D-mannonate-6-phosphate. It seems probable that in both the chemical and the biological synthesis 2-deoxy-D-gluconate-6-phosphate is formed initially and is then immediately converted to D-mannonate-6-phosphate. The production of 2-deoxy-D-gluconate-6-phosphate by cells has not previously been reported although cell-free extracts of *Leuconostoc mesenteroides* have been found[5] to contain an enzyme which catalyses the reduction of triphosphopyridine nucleotide by 2-deoxy-D-glucose-6-phosphate. The relative intensity of blackening of the two spots on our radioautographs indicates that although D-mannonate-6-phosphate is formed, most of the 2-deoxy-D-glucose is converted only to 2-deoxy-D-glucose-6-phosphate.

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An Antibiotic Complex derived from a *Streptomyces* and active against Gram-positive Bacteria

Culture medium in which a *Streptomyces* (Strain No. 1415 of the Montecatini collection; now listed as No. 3838 of the collection of the Institute of Microbiology, Rutgers University) had been grown was found to contain an antibiotically active complex of eight components, effective against Gram-positive bacteria.

The organism in question produced pinkish growth with a diffusible pink pigment. The spores were spherical to ovoid, and the sporophores in the form of tufts of short hyphæ with slightly curled ends.

It was capable of hydrolysing starch and decomposing gelatin and casein.

Cultures for antibiotic production were made in a medium containing (per cent w/v) 0·25 peptone, 0·25 meat extract, 1·0 glucose, 0·5 sodium chloride and 1 per cent (v/v) corn steep liquor (40 per cent solids), in a 20-litre tank at 27° C. These were grown for 72 hr., constantly stirred, and aerated with 15–20 litres/min. of air.

The medium was then filtered and extracted with ethyl acetate, the solvent dried, reduced *in vacuo* and washed in dilute sodium hydroxide. The ethyl acetate was further reduced in volume and passed through a short column of an intimate mixture of 'Darco G 60' and 'Hyflo-Supercel' (1 : 1). After elution with ethyl acetate and concentration, the crude complex was precipitated by petroleum ether, dissolved in hot carbon tetrachloride and passed through a similar, longer column, eluted with benzene and again precipitated by petroleum ether.

The purified complex was a white, amorphous powder, soluble in lower alcohols, acetone, chloroform and ether, insoluble in water. The percentage composition was approximately: C, 59·5; H, 8·0; N, 2·0 ($c = 1$ per cent in methanol) $[\alpha]_D^{20} = -34·5°$. In the hydrolysate, seven amino-acids were detected: glycine, proline, alanine, valine, phenylalanine, leucine and aminobutyric acid (probably the γ-isomer). The ultra-violet adsorption spectrum showed a maximum at 282 mμ.

By paper chromatography, eight components of the complex could be recognized.

The *LD*50 in albino mice was 400 mgm./kgm. intravenously, 600 mgm./kgm. intraperitoneally and 2 mgm./kgm. subcutaneously.

The complex was active against *Bacillus* spp., staphylococci and hæmolytic streptococci at 0·25 μgm./ml. or less, but was inactive against Gram-negative bacteria and non-hæmolytic streptococci.

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PHYSIOLOGY

Effect of Hydration on Hexobarbitone-induced Sleep in Mice

Hydration of laboratory animals and man produces a complex picture generally indicating increased excitability of the central nervous system. This is attributed to osmotic disturbances produced by fall of the extracellular electrolytes[1]. It is therefore interesting to find in albino mice that after hydration the sensitivity of the central nervous system to hexobarbitone was increased simultaneously. This conclusion is based on the results obtained from the following experiments.

Adult albino mice received intraperitoneally hexobarbitone sodium 100 mgm./kgm. after water-load. Control mice received only hexobarbitone. In some control experiments mice received water (or normal saline or 5 per cent glucose) just after recovering from sleep (Table 1).

About 2–4 hr. before becoming absorbed, intraperitoneally administered 5 per cent glucose solution is known to remove electrolytes from the extracellular body fluid, thus producing a condition comparable to hydration[2]. Mice receiving this treatment were used ½ and 2 hr. later for hexobarbitone sleep. The effect of normal saline was also studied (Table 2).

Table 1. Effect of Hydration on Hexobarbitone Sleeping Time in Mice. Water at 37° C. injected intraperitoneally. Animals were kept at 32-35° C.

Hydration mil. % of body-weight	Min. between hydration and hexobarb.	Sleeping time min. ± *S.D.*	No. of mice used	Value of *P* (*t* test)
Nil	Nil	21 ± 4·1	20	
5	30	18 ± 6·8	21	*
10	0	36 ± 9·5	12	< 0·01
10	15	56 ± 12·7	15	< 0·01
10	30	49 ± 11·2	20	< 0·01
10	60	40 ± 12·3	15	< 0·01
10	240	26 ± 6·8	8	< 0·05
10	Just after hexobarb. sleep	22 ± 5·5	20	

* Not significant.

Table 2. Effect of 0·9 per cent Sodium Chloride Solution or 5 per cent Glucose at 37° C., injected intraperitoneally, on Hexobarbitone Sleeping Time in Mice. Room Temperature 32 – 35° C.

Fluid mil. % of body-weight	Min. between fluid and hexobarb.	Sleeping time min. ± *S.D.*	No. of mice used	Value of *P* (*t* test)
10 (Glucose)	30	25 ± 5	18	< 0·02
10 (Glucose)	120	54 ± 12·9	18	< 0·01
10 (Saline)	30	22 ± 8·8	18	*
10 (Saline)	120	17 ± 8·4	12	*

* Not significant.

Results indicate that 10 per cent but not 5 per cent hydration prolongs hexobarbitone sleeping time, the maximal effect occurring 15 min. after hydration. The effect was also seen 30 and 60 min. after hydration. The rate of water excretion can explain these results. Animals recovering from sleep go back again to sleep after hydration but not after saline load. This suggests sensitization of the central nervous system and excludes other mechanisms of action. Hydrated mice were quieter but essentially normal in their behaviour. All experiments could be repeated on the same animals after 2–3 days.

Glucose solution, requiring a certain time-interval to deplete the extracellular electrolytes, produces a distinct effect 2 hr. later. Further work is in progress.

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Evidence for the Identity of Natriferin, the Frog Water-Balance Principle and Arginine Vasotocin

Maetz, Morel, and Lahlouh[1] recently reported that pituitary extracts from amphibians and teleosts contain a principle which promotes the active transport of sodium by the isolated skin of the frog *Rana esculenta*. This factor cannot be oxytocin or a vasopressin since it has approximately ten times more activity on sodium transport than do mammalian extracts of similar oxytocic activity. Maetz *et al.* named this principle 'natriferin'.

Heller[2,3] first pointed out that fish, amphibian, avian, and reptilian pituitary extracts exert far more effect on frog water-balance than can be attributed to their content of oxytocin or vasopressin. Pickering and

Table 1. Relative Activities of Oxytocin, Arginine Vasopressin, Arginine Vasotocin, and a Bull-frog Neurointermediate Lobe Extract on Natriferin Assays, and on other Assays (refs. 5, 8, 9, 12)

Preparations	Activities (μgm./mgm. dry weight)				
	Rat vasopressor	Rat uterus (no Mg^{++})	Frog bladder	Frog antidiuretic	Frog natriferic
Bullfrog neurointermediate lobe	0·52 (0·50–0·54)	0·59 (0·55–0·64)	79	190	47
Arginine vasotocin	64 (62–67)	37 (34–41)	10,000	19,000	4,400
Arginine vasopressin	300	9	70	—	70
Oxytocin	—	420	360	360	360

The figures shown in parentheses are the 95 per cent confidence limits.

Heller[4] have recently succeeded in separating the 'water-balance principle' by paper chromatography of fish, frog, and fowl pituitaries. From chromatographic and pharmacological studies on the chicken neurohypophysis Munsick *et al.*[5] conclude that this gland contains oxytocin and a peptide pharmacologically indistinguishable from arginine vasotocin (arginine[8] oxytocin). This analogue of the mammalian neurohypophysial hormones has been synthesized by Katsoyannis and du Vigneaud[6,7] and generously supplied by them for study in this Department. It has much greater activity on the water permeability of the frog bladder than any other neurohypophysial hormone or analogue that I have yet tested[8]. It is also extremely potent in causing water uptake (water-balance response) and antidiuresis in frogs[9]. I have gathered pharmacological evidence for its existence in pituitary extracts from cyclostomes, teleosts, amphibians, reptiles, and birds[10]. It is probably not present in elasmobranchs or mammals.

Natriferin has been clearly demonstrated in teleosts and amphibians, but appears to be absent in mammals and elasmobranchs[1]. The correspondence of the phyletic distributions of natriferin, the water-balance principle, and what I believe to be arginine vasotocin strongly suggests that they are identical. It is of pressing interest, therefore, to assay synthetic arginine vasotocin for 'natriferic' activity.

I have used a simplified Ussing–Zerahn[11] short-circuited frog skin for natriferic assays. An inside-out skin bag from the leg of *Rana pipiens* is suspended in a carbon dioxide–bicarbonate-buffered Ringer's solution. The bag is filled with the same solution. 'Sodium current' is measured by balancing the electromotive force across the skin and recording the applied current. Extracts and synthetic peptides are added to the bath bathing the physiological inside (dermal surface) of the skin. The maximum increase in sodium current recorded in the 15-min. period following the addition is taken as the response. Four-point balanced assays are performed. Synthetic oxytocin ('Syntocinon', Sandoz) is used as standard. Relative potencies are expressed in terms of units, one unit of natriferic activity being defined as the natriferic activity of one oxytocic unit of oxytocin. Calculations are based on the assumption of a linear regression of response on the logarithm of dose.

The results of natriferic assays on a bull-frog (*Rana catesbiana*) neurointermediate lobe extract, on synthetic arginine vasotocin, and on arginine vasopressin are shown in Table 1. There are wide variations of the values obtained for natriferic activity of arginine vasotocin and frog extract on different assays (the figures shown are geometric means of the results of five assays on each preparation). The variation appears primarily to be due to a 20-fold difference in the doses of oxytocin that must be used to obtain good responses in different frog skins. The sensitivity of the skins to frog extract or arginine vasotocin remains much more constant among different frogs. If one compares the natriferic values obtained when both frog extract and arginine vasotocin are assayed on the same skin there is a much more constant relationship between their natriferic activities. This may be taken as additional evidence in support of the contention that frog extract contains arginine vasotocin.

Activities of arginine vasopressin, oxytocin, bullfrog neurointermediate lobe extract and arginine vasotocin on other selected assays are also shown in Table 1. It appears that arginine vasotocin could, if present in the bull-frog neurointermediate lobe in a concentration of approximately 8 μgm./mgm. dry weight, account for the vasopressor, frog bladder, frog antidiuretic, and frog natriferic activities of the extract. The high oxytocic activity of the frog extract is believed to indicate that oxytocin is also present[10].

It appears probable that both natriferin and the frog water-balance principle are arginine vasotocin. It would not appear necessary, therefore, to ascribe the natriferic activity of frog and teleost pituitary extracts to a new hormonal principle[1] unless there is commanding evidence that such exists distinct from principles previously described.

Further work on the relationships between peptide structure and natriferic activity are planned. It is anticipated that this response will show a high degree of specificity as does the frog bladder response[8]. The presence of natriferic activity in neurohypophysial extracts from vertebrates other than those investigated by Maetz *et al.*[1] should also be investigated.

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Effect of Reserpine on the Nucleotide and Catecholamine Content of the Denervated Adrenal Medulla of the Rat

Reserpine causes a depletion of the catecholamines and adenosine triphosphate stored in the amine granules of the adrenal medulla[1–3]. This effect may, however, have been produced indirectly through the secretory nerves[3]. Such a stimulation of the medulla induced by insulin hypoglycæmia or morphine causes a concomitant release of amines and adenosine triphosphate[4,5]. The essential problem, however, is how reserpine causes amine depletion by direct action on the cells. As a preliminary attempt to elucidate this problem, the effect of reserpine on the nucleotide and amine content of the denervated rat medulla was studied.

The left adrenal medulla of twelve male rats (body-weight 350–400 gm.) was denervated by cutting the splanchnic nerves. This produces a total denervation[7]. After ten days the animals were divided into three equal groups. Reserpine (10 mgm./kgm. subcutaneously) was administered to each of the rats. The animals were killed 24 hr. after the injection and the adrenal medullæ were dissected, extracted and analysed for catecholamines and adenosine phosphates by means of high-voltage paper electrophoresis in the way described in a previous paper[6]. This method yields values for the adenosine phosphates which are somewhat higher than the real ones owing to the presence of other nucleotides[3]. Each extract contained the four left or right medullæ from the rats in each group. The rats were of the same weight and stock as those used in an experiment made at the same time. The normal animals in that work[6] were therefore taken as controls. Denervation of the medulla does not produce any certain changes of the nucleotide or amine content[6].

The results are presented in Table 1.

Table 1. μMOLES in Medullæ from 4 Glands

		Catechol-amines	ATP	ADP	AMP
Normal glands	Mean	0·365	0·0845	0·0315	0·0096
Reserpine Denervated glands	I	0·280	0·0650	0·0255	0·0077
	II	0·190	0·0415	0·0225	0·0061
	III	0·230	0·0550	0·0215	0·0070
	Mean	0·235	0·0540	0·0225	0·0069
		−36%	−36%	−29%	−28%
Non-denervated glands	I	0·110	0·0245	0·0175	0·0063
	II	0·0570	0·0140	0·0115	0·0048
	III	0·0915	0·0200	0·0130	0·0052
	Mean	0·0860	0·0195	0·0140	0·0054
		−76%	−77%	−56%	−44%

Reserpine produced an amine depletion which was on an average 77 per cent in the normal glands and 36 per cent in the denervated ones. As previously found for normal medulla in fowl[1], rat[2], and sheep[3], disappearance of a corresponding amount of adenosine triphosphate occurred in both the normal and the denervated glands. The adenosine monophosphate and adenosine diphosphate content also decreased, but to a lesser extent.

Thus it seems that the release of amines from the adenosine triphosphate amine storage complex produced by reserpine through direct action on the cells is accompanied by a release and/or breakdown of an equivalent amount of adenosine triphosphate which, furthermore, does not lead to an accumulation of adenosine diphosphate or adenosine monophosphate. In this respect the direct reserpine action and the action of nervous secretory stimuli give a similar effect. This may mean that both reserpine and secretory stimuli act through the same mechanism (for example, activation of a release or inhibition of a storage mechanism). Considering the structure of the adenosine triphosphate amine storage complex[8], it seems probable, however, that an amine release—irrespective of the way in which it is brought about—must always be accompanied by a corresponding release (or breakdown) of adenosine triphosphate. The findings, therefore, only seem to imply that the amines released from the granules by reserpine do not accumulate to any appreciable extent in the cytoplasmic sap.

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Effect of Three Compounds related to Diethylstilbœstrol on the Phagocytic Activity of the Reticulo-Endothelial System

It has been reported previously that diethylstilbœstrol is a strong stimulant of the phagocytic activity of the reticulo-endothelial system[1]; that the minimum dose required to stimulate the phagocytes is about 100 times greater than that necessary to induce œstrus[2]; and that after treatment with 0·5 mgm. diethylstilbœstrol daily for six days the phagocytic activity returns to within normal control limits in about eight days[3].

The present communication is a report of the action on the phagocytic activity of the reticulo-endothelial system of three compounds related to diethylstilbœstrol.

Twenty-five male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used in these experiments. Five animals were used to test each compound, each animal receiving one subcutaneous dose of 0·5 mgm. of the compound in 0·05 ml. arachis oil daily for six days. On the eighth day after the commencement of the treatment the phagocytic activity was measured by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[4], the procedure used being that described in previous communications[1], and the total-body phagocytic activity or phagocytic index being denoted by the symbol K.

Ten of the animals were used as controls and were given 0·05 ml. arachis oil once daily for six days. The phagocytic activity was then measured on the eighth day by the carbon method referred to above. The arachis oil controls showed a phagocytic index or K value of $13 \pm 2{\cdot}4$.

The results are shown in Table 1. The three compounds are active œstrogens, and it can be seen that all of them are strong stimulants of phagocytic activity. These results again suggest that the configuration of the molecule which confers œstrogenic activity is also essential for reticulo-endothelial stimulation. In addition, the results show that phagocytic stimulation

Table 1. Effect of Three Compounds related to Diethylstilbœstrol on the Phagocytic Activity of the Reticulo-Endothelial System

Compound used	Phagocytic index (*K* value)	Weight of liver (gm.)	Weight of spleen (gm.)	Œstrogenic activity (R.U.)
Diethylstilbœstrol monomethyl ether	85 ± 31	1·79 ± 0·11	0·13 ± 0·01	0·0025 mgm.
Diethylstilbœstrol dimethyl ether	88 ± 19	1·86 ± 0·17	0·15 ± 0·04	0·02 mgm.
4-Hydroxy-α: β-diethyl dibenzyl	74 ± 14	1·45 ± 0·14	0·13 ± 0·03	0·04 mgm.
Arachis oil controls	13 ± 2·4	1·14 ± 0·17	0·11 ± 0·03	

is accompanied by a corresponding enlargement of the liver and spleen. These are the principal reticulo-endothelial organs and it has been recorded previously that about 90 per cent of the carbon is removed from the blood by the liver, about 3 per cent by the spleen and the remainder by the phagocytes of the lymph nodes, connective tissue and bone marrow[5]. It was also found that the enlargement of liver and spleen is not due to water retention, but appears to be the result of hypertrophy of hepatic and splenic tissue.

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Infra-red Examination of the Association of Cornea Proteins with Water

Water, the most abundant molecular constituent of the body, is the necessary polar solvent in which physiological and biochemical units associated with cellular biology occur. It forms hydrogen bonds with cellular components which contain polar structural groups such as the protein collagen[1] found in the cornea. Direct evidence for molecular perturbations that occur during change in water content in intact tissue has been lacking, although changes are believed to occur. The present work is concerned with the demonstration and interpretation of the changes that occur in the infra-red spectra of the ox cornea during dehydration and during attempts at re-hydration.

The tissue chosen for investigation was the ox cornea, which has a water content of 75–80 per cent with collagen making up about 70 per cent of the solids. Corneas were excised 1–3 hr. after the animals were slaughtered and then stored at 4° C. until ready for use. They were dehydrated by exposure to infra-red radiation during the runs, which usually lasted ½ hr. each, and they were allowed to dry between runs at room temperature in a desiccator. The Perkin-Elmer model 12 infra-red spectrometer with a sodium chloride prism was used for recording the spectra of the cornea, which was mounted in the slit position. The region of the spectra covered was 1·2–2·7μ.

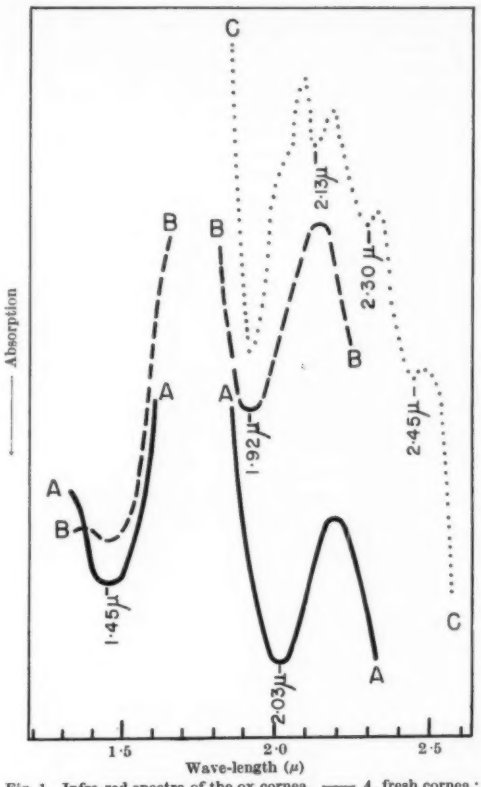

Fig. 1. Infra-red spectra of the ox cornea. —— *A*, fresh cornea; – – – *B*, 5-hr. specimen; · · · · *C*, 2-day specimen

Progressive stages in drying of the ox cornea are shown in Fig. 1. Curve *A* represents the spectral absorption curve immediately after excision. The spectra after 5 hr. and 2 days of drying are indicated by curves *B* and *C*, respectively. Immediately after excision, the maximum absorption bands were obtained at 1·45μ and 2·03μ. After dehydration for 5 hr., the wave-lengths of the absorption bands were 1·45 and 1·92μ, and after two days, they were 1·92, 2·13, 2·30 and 2·45μ. The absorption at 1·45μ in curves *A* and *B* of Fig. 1 is ascribed to water, which decreases between curves *A* and *B*. The absorption band in curve *A* at 2·03μ has been eliminated in the 5-hr. specimen (curve *B*) although water is known to be present since there is a residual absorption at 1·45μ. After 5 hr. the background absorption of the corneal protein is sufficiently intense in the 2μ region to obscure the absorption of the small amount of water present.

Attempts to rehydrate the corneas which had been dehydrated for 2 days were carried out by dampening with water and immersing in water for 30 min. and 2 days. In each case, there is an increase in absorption at 1·45μ and 1·92μ, which are approximately the absorption bands of water. The combined absorption curves of the 2-day-old specimen and that of water do not correspond to the original spectrum shown in Fig. 1, curve *A*. A number of determinations were made, and reproducibility was obtained for the initial and final runs.

The infra-red absorption curve obtained with dehydrated cornea to which water had been added

does not correspond to the absorption spectrum of the original tissue. It is apparent that the withdrawal of water from the tissue leads to structural changes of the cornea, which are revealed by the infra-red absorption bands found in the dried tissue. The inability to re-establish the infra-red curve *A*, Fig. I, by adding water to the dried tissue indicates that the polar groups which were associated with water in the fresh cornea are no longer available. The loss of water in the cornea apparently breaks the association between the protein and water molecules. Since the final dried cornea is hard and has the physical characteristics of a high molecular weight polymer, it is suggested that the active polar groups of the released protein may then become re-oriented among themselves to form higher molecular weight components.

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Diuretic Activity of 2-Amino-4-meta-chloro-anilino-1,3,5,triazine (*R*-37)

A series of new derivatives of *s*-triazine synthesized in the Department of Chemotherapy, Haffkine Institute, Bombay, have been tested for their diuretic activity. Of the 12 compounds tested, 2-amino-4-meta-chloro-anilino-1,3,5,triazine (*R*-37), which is the metachloro analogue of amanozine, was found to be most promising. Diuretic activity was determined by a modified method of Lipschitz *et al.* (1943) developed in this laboratory[1].

Unanæsthetized rats received normal saline orally, 2·5 per cent of body-weight, followed by the drug under test. Urine was collected over a period of 5 hr. Dogs were anæsthetized, hydrated with normal saline, 2·5 per cent of body-weight, and the drug given intravenously. Urine was collected for the next 5 hr. by catheterizing both ureters, and urinary sodium, potassium and chloride were determined.

Results obtained with *R*-37 were compared with those obtained with urea and hydrochlorothiazide in rats, and with hydrochlorothiazide in dogs. The results of experiments with rats are given in Table 1, and those with dogs in Table 2.

Table 1. Results for Rats

Average of percentage excretion of initial load of water, sodium and chloride in groups of four animals. Potassium values represent average of total amount of potassium thrown out by each group

	Urea	Hydrochlorothiazide		*R*-37	
Dose (mgm./kgm.)	750	1·25	2·58	5·0	10·0
Groups	(14)	(8)	(8)	(8)	(8)
Sodium	30	80*	89	60*	154
Chloride	111	217	211	157	287
Water	61	71	64	58	122
Potassium	0·50	0·76†	0·90*	0·64	0·77*†

A 't' test for significance was carried out. Any two values not underscored by the same line or marked with same signs are significantly different (*P*, 0·01).

Table 2. Results for Dogs

Average of percentage increase over control values of urine volume and electrolyte excretion over intravenous administration of the drug: control observations are for 1 hr., while 'test' values are average of 5 hr. in each animal. Each figure is an average from three animals

Drug	Hydrochloro-thiazide	Hydrochloro-thiazide	*R*-37	*R*-37
Dose (mgm./kgm.)	2·5	5·0	5·0	10·0
Sodium	270	1,590*	100	1,130*
Potassium	315*	280*	55	360*
Chloride	390	1,135	265	835
Urine volume	470	225	230	1,110

A 't' test for significance was carried out. Any two values not underscored by the same line or marked with asterisks are significantly different (*P*, 0·01).

These results show that *R*-37 is a very powerful diuretic in animals and compares favourably with hydrochlorothiazide in the range of dosages of the two drugs employed by us. The drug has a good margin of safety in animals and is not toxic when used in diuretically active dosage given daily over a period of four weeks in animals. None of the dogs given *R*-37 in doses of either 5 mgm./kgm. (3 dogs) or 10 mgm./kgm. (3 dogs) showed any sign of hæmaturia or crystalluria.

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A Suggested Effect on Levels of Catecholamine in Brain produced by Small Doses of Reserpine

In a recent communication, Sheppard and Zimmerman[1] criticized the results obtained by numerous other workers indicating that reserpine reduces the content of brain norepinephrine and serotonin. Their objections were based on the "toxicological doses" which had been used. However, they failed to mention that reserpine had also been reported to reduce levels of brain serotonin[2] and norepinephrine[3] in non-toxic doses as low as 0·1 mgm./kgm. and to deplete heart norepinephrine in doses as low as 5 μgm./kgm.[4]. Sheppard and Zimmerman's results showed that the administration of 0·1 mgm./kgm. of reserpine subcutaneously to female guinea pigs weighing 300 gm. elicited a rise of 74 per cent in brain norepinephrine in 20 min. and a rise in heart norepinephrine of 30 per cent in 20 min. and 45 per cent in 2 hr. No diminution in brain and heart norepinephrine was noted for 8 hr. In contrast, serotonin-levels in the brain fell by 50 per cent in 4 hr. They concluded that their results constituted the first demonstration of a rise in catecholamine content following the administration of reserpine.

Sheppard and Zimmerman measured norepinephrine fluorometrically by a procedure using a filter for isolating the activation and fluorescent light bands. Since filters have relatively wide spectral bands, the validity of their values is contingent on proof that the method for norepinephrine is specific. This is especially relevant in view of the presence of reserpine and its metabolites in the body. However, no evidence is offered for the specificity of the method.

We have repeated the experiments using a fluorescent spectrofluorometer which permits use of narrow spectral bands and methods of proved specificity for norepinephrine[5] and serotonin[6]. The administration of 0·1 mgm. of reserpine per kgm. subcutaneously to female guinea pigs weighing about 300 gm. at no time caused a rise in brain or heart norepinephrine. In 20 min., when Sheppard and Zimmerman had found a 74 per cent rise in brain norepinephrine, we found that serotonin and norepinephrine showed no change from normal, or perhaps a small decline. After 4 hr. the brain amine-levels were 60–70 per cent of normal (Table 1).

Table 1. GUINEA PIG BRAIN AND HEART AMINES AFTER ADMINISTRATION OF 0·1 MGM./KGM. OF RESERPINE SUBCUTANEOUSLY

		γ/gm.* ± S.D.	Percentage of normal
Brain serotonin	Control	0·28 ± 0·04	100
	Reserpine, 20 min.	0·28 ± 0·02	100
	Reserpine, 4 hr.	0·19 ± 0·04	68
Brain norepinephrine	Control	0·42 ± 0·04	100
	Reserpine, 20 min.	0·40 ± 0·02	95
	Reserpine, 4 hr.	0·25 ± 0·07	60
Heart norepinephrine	Control	2·71 ± 0·34	100
	Reserpine, 20 min.	2·46 ± 0·38	91
	Reserpine, 4 hr.	0·63 ± 0·30	23

* Each value represents the average from 6 animals.

Heart norepinephrine fell to 90 per cent of normal in 20 min. and was only 20 per cent of normal in 4 hr. (Table 1).

These results show that the fall in brain and heart amines after the administration of small doses of reserpine to female adult guinea pigs is qualitatively similar to that observed in other animal species when using larger doses of reserpine.

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THE preceding communication contains data refuting the rise in catecholamines which we had observed 20 min. after the subcutaneous injection of reserpine (0·1 mgm./kgm.). In repeating the experiment in order to check the questioned specificity of the analytical method, we found that we were unable to reproduce our original findings. Instead, the brain catecholamine-levels expressed as percentage of controls were found to be 105, 100, 64, 26, 37 and 31 per cent after ¼, 2, 4, 6, 8 and 72 hr. The shape of the curve was very much like that observed for serotonin and the 4-hr. values agreed with those reported by Orlans and Brodie. We are, at present, unable to detect the factors in the original experiments which gave us the values we reported, but we intend to pursue this matter further. In spite of this discrepancy, the serotonin values remain undisputed and the main conclusion remains valid, namely, that there exists no correlation between the levels of brain amines and the intensity of observable effects.

It is unfortunate that Orlans and Brodie, while correctly questioning the method of analysis for catecholamines, misinterpreted and mis-stated what was written in our original letter. Suffice it to say that our communication was not a criticism of the many skilled workers who had demonstrated the ability of reserpine to lower tissue amine-levels. Contained in it, however, was a criticism of the attempts by some workers to attribute the presumed central effects of large doses of reserpine (1–5 mgm./kgm.) to changes in the levels of brain serotonin or norepinephrine. The actions of reserpine described by Brodie *et al.*[1] as "loss of righting reflex, enhanced salivation and active closure of the eyelids" would be considered as undesirable toxic side-effects if found to occur clinically. To imply that such effects describe in equal fashion such terms as tranquillizing, sedative and central action of reserpine does little to clarify a terminology which is already too vague for scientific usage. It is important to define terms more clearly and to signify what responses one is trying to correlate with changes in tissue amine-levels. It remains to be determined which, if any, of the effects of reserpine may be attributed to changes in serotonin or norepinephrine-levels alone. Previous work with reserpine labelled with carbon-14 or tritium[2] had demonstrated that the alkaloid was present in the brain during the entire period of visible action of the drug, but that here too no correlation could be made between the intensity of response and drug-level. It is important to recognize that the application of other techniques as used in the study of behaviour may bring to light some actions of the drug which are not readily observed, and for which a correlation can be found with changes in brain amine and/or reserpine-levels. In our opinion, no experiment has been reported which conclusively eliminates reserpine, *per se*, as the active agent and establishes serotonin and/or norepinephrine in its place. It remains for future work to establish the true mechanism of action of reserpine and similar alkaloids.

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HÆMATOLOGY

β-Globulin Polymorphism in Chimpanzees

INTRASPECIFIC variation in the electrophoretic mobility of serum iron-binding β-globulins ('transferrins') have been described in man[1,2], cattle[3–5], sheep[6,7], goats[8] and certain monkeys[4,9]. In all but monkeys these variations have been established as inherited.

We have recently examined the sera of 25 chimpanzees, *Pan satyrus*, and observed seven β-globulin phenotypes. Prior to our examination, these animals had been used for the assay of poliomyelitis vaccine. Fifteen were affected with spontaneously acquired tuberculosis. Serum was held 2–20 weeks at − 15° C. before use. Electrophoresis on borate-

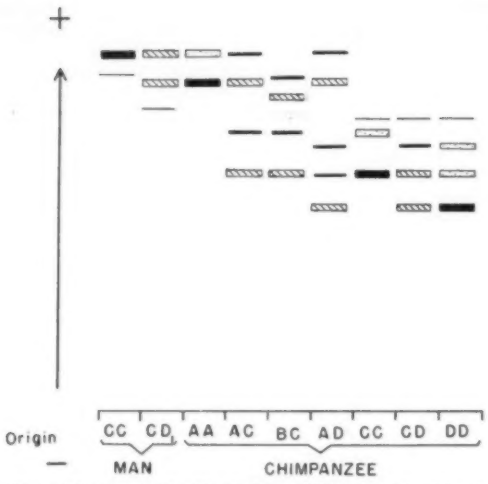

Fig. 1. Diagram showing the relative mobilities and intensities of iron-binding β-globulins in various phenotypes of man and chimpanzees. Order of decreasing intensity is as follows: black bar, hatched bar, stippled bar, thin bar, line

Table 1. Frequency of β-globulin ('Transferrin') Phenotypes in Chimpanzees

Phenotype	No. of animals in which phenotype was observed
AA	1
AC	3
BC	4
AD	5
CC	9
CD	1
DD	2
	25

buffered hydrolysed starch gel was performed according to the technique described by Smithies[10]. Use of gel buffered with *tris*(hydroxymethyl)aminomethane[11] yielded less-satisfactory separation. The serum β-globulin of each animal was saturated *in vitro* with iron-59 (ferrous citrate)[2], a constant voltage of 4–5·5 V./cm. applied for 18–21 hr., and radioautographs obtained[3]. The serum of each animal was compared repeatedly with those of other animals. A diagram of observed transferrin types is shown in Fig. 1 and the frequency of these types in Table 1.

The inheritance of the observed phenotypes is unknown and likely to remain so because of the difficulty attendant on mating these animals in captivity. The designation of phenotypes as shown is thus entirely arbitrary. It seems possible, however, that the β-globulin phenotypes AA, CC and DD represent homozygous states. These phenotypes exhibit two to four bands the intensity of which is inversely related to mobility and in these respects are similar to cattle homozygous at the β-globulin locus[5]. Moreover, the *in vitro* mixture of equal parts of AA and CC sera results in an electrophoretic appearance indistinguishable from AC serum, suggesting that AC animals are hybrid at the β-globulin locus. Similarly, mixtures of phenotypes AA–DD and of phenotypes CC–DD are indistinguishable from AD and CD phenotypes, respectively. A BB phenotype was not observed although its existence might be predicted by the occurrence of BC sera.

The presence of faintly labelled zones of iron-binding β-globulins was overlooked in preliminary experiments, and thus there may be numerous additional zones, characteristic of each phenotype, which cannot be detected by the technique employed. The appearance of multiple β-globulin zones in homozygotes has been observed in a variety of animals[4–9] and may occur in man. Indeed, several human sera of Tf-CC type frequently exhibited, as shown in Fig. 1, a faintly labelled band lying midway between the position of C and D_1 in man. The serum of one CD_1 individual, a Negro woman with systemic lupus erythematosus, was repeatedly examined and exhibited the faintly labelled slow-moving band indicated in Fig. 1. Such bands may, of course, be artefactual in nature.

One explanation for a multiplicity of 'transferrin' zones may lie in the polymerization of some parent protein unit. Such a hypothesis appears quite likely in the case of human haptoglobins[12].

It was noted in passing that the haptoglobin of each chimpanzee, when fully saturated with human hæmoglobin A, appeared as a single band lying in approximately the same position as human haptoglobin *Hp* 1–1. Some animals with gross anatomical evidence of pulmonary tuberculosis had haptoglobin hæmoglobin binding capacities in excess of 300 mgm. per cent, while others with the same disease had less than 30 mgm. per cent[13].

We wish to thank Dr. David Bodian for providing the chimpanzees studied and Dr. Neal Nathanson for his assistance.

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Effect of Ribonuclease on Erythrocyte Antigens

Results reported earlier from this laboratory suggest that the Rh and Lutheran human blood-group antigens owe at least some portion of their specificity to ribonucleic acid derivatives[1,2]. These observations were later confirmed by Allen (personal communication). Boyd, McMaster and Waszczenko-Zacharczenko[3] reported that they could not confirm the results cited above. However, more recently Boyd (personal communication) has observed an inhibitory effect reported earlier for these ribonucleic acid

derivatives. This hypothesis has been further tested by treating erythrocytes with the enzyme ribonuclease. (Two samples from Worthington and one from Nutritional Biochemicals were used.) If, indeed, any antigenic specificity is due to ribonucleic acid derivatives, then treatment with this enzyme should remove these, at least partially, from the cells, and thus lower the agglutinability of these cells with the antibodies specific for the affected antigens. On the other hand, those antigens which are presumed not to involve ribonucleic acid derivatives should be unaffected by this treatment.

Table 1

Antibody	Sample No.	Titration scores: Treated with ribonuclease	Titration scores: Untreated (saline control)	Enzyme concentration (mgm./ml.)	Time (hr.)
Anti-A	1	85	85	2	2
	2	91	91	2	4
	3	93	93	2	4
	4	88	88	2	11
Anti-B		88	88	2	4
Anti-M	1	47	48	2	2
	2	60	59	2	4
Anti-N		53	53	4	4
Anti-P		12	12	2	4
Anti-I	1	36	36	2	2
	2	35	33	2	2
Anti-Lu[a]		16	28	4	4
Anti-Lu[b]		12	30	4	4
Anti-D	1	41	56	2	2
	2	30	56	2	2
	3	45	69	4	4
	4	50	71	4	11
Anti-C	1	52	72	2	2
	2	51	62	2	11
	3	52	64	4	4
	4	27	48	2	2
	5	21	48	2	2
Anti-E		29	47	2	2
Anti-c		13	25	2	2
Anti-e	1	43	59	2	2
	2	38	50	2	4

Samples of packed cells were incubated for varying times, as is shown in Table 1, with the enzyme ribonuclease. The enzyme concentration was 2 mgm./ml. or 4 mgm./ml., and ten volumes were used with a single volume of cells. Identical controls were set up using saline instead of ribonuclease. After incubation with either ribonuclease or saline, the cells were washed, and matched cell suspensions prepared. These were titrated with the various antisera shown in Table 1, and scored on the Race scale[4]. Only saline agglutinins were used.

With anti-A, -B, -M, -N, -P and -I, as can be seen in Table 1, there is no appreciable difference in titration scores between the cells treated with ribonuclease and the controls incubated in saline. However, with the anti-Rh and the anti-Lutheran antibodies, the agglutinability of the treated cells is apparently much less than the untreated controls. While not attempting here to evaluate optimum time or enzyme concentration for the reaction, it seems clear that under all sets of conditions tested, the treated cells have lost part of their Rh and Lutheran specificities at the same time as the other antigens tested for were unaffected. Since the activity of the enzyme, ribonuclease, is highly specific in that it acts only on polyribonucleic acids, it seems reasonable to conclude that the loss of ribonucleic acid components from the cell caused the lowering of agglutinability of the cells. This, added to the evidence of inhibition of agglutination cited above, would suggest still more strongly that there are one or more ribonucleotide components of the Rh and Lutheran antigens.

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BIOLOGY

A Bleached Variant of *Euglena gracilis* showing a Doubling of the Content of Deoxyribonucleic Acid

WHEN certain strains of the green flagellate *Euglena gracilis* are grown at 34–35°, cultures devoid of chloroplasts are obtained[1]. These cells, which we term bleached, resemble the normal organisms cultivated in the dark (designated colourless), but they have lost a hereditary character necessary for the formation of the plastids[2]. In the course of a study of the chemical changes attending the loss of the photosynthetic apparatus in *Euglena*, a bleached culture ('Bleached II') derived from the normal strain *z* by thermal treatment, which had been carried through frequent liquid transfers for about three years, was found to contain twice the amount of deoxyribonucleic acid that is present in the original green or colourless cells. A comparison of the properties of this bleached variant with those of colourless cells showed the first to contain also more ribonucleic acid and protein and to have a slower rate of growth. Although the nuclei of these two cell types, when treated by the Feulgen method, appeared of equal size, those of the bleached organisms were coloured more intensely.

In an attempt to study this phenomenon in more detail, we produced clones of bleached cells by cultivation either at 34° or in the presence of streptomycin (10 mgm./ml.)[3]. These newly produced bleached organisms ('Bleached I') contained, to our surprise, an amount of deoxyribonucleic acid identical with that of the normal cells; nor did the further cultivation of these cells at 34° for several weeks with frequent transfers bring about an alteration in the normal nucleic acid content. Clones obtained from spontaneously occurring bleached cells also were found to contain the normal quantity. Table 1 records the contents of nucleic acid and protein found in the colourless cells and the two types of bleached organisms. The quantity of deoxyribonucleic acid present in green cells grown in the light is the same as that found in the colourless organisms.

It would appear that the doubling of the content of deoxyribonucleic acid in the bleached strain of type II constitutes a newly acquired and stable hereditary characteristic that may be described tentatively as an instance of experimentally induced polyploidy or polyteny. A change in the timing of the duplication of deoxyribonucleic acid during the interphase could, however, also simulate an alteration

Table 1. Properties of Colourless and Bleached Cells of *Euglena gracilis*, Strain *z**

Type of cells	Deoxyribonucleic acid (μmol. per 10^7 cells)	σ	Ribonucleic acid (μmol. per 10^7 cells)	Protein (mgm. per 10^7 cells)	Generation time (hr.)
Colourless	0·09	0·02	0·60	2·1	7·3
Bleached I	0·11	0·02	0·67	2·2	8·0
Bleached II	0·20	0·04	0·80	3·3	8·8

* The cells were grown in the medium described previously (ref. 4). The chemical analyses were performed on large cell samples taken from cultures in the late logarithmic phase. The nucleic acids were determined by a modification, to be described later, of the Ogur–Rosen method (ref. 5); protein was estimated by means of the biuret procedure (ref. 6). The standard deviation is given as σ. The generation time was measured in cultures in the early logarithmic phase.

of the cellular content of this substance; and in the absence of a detailed cytochemical study this possibility cannot be excluded entirely. Our observations on the bleached cultures of type I demonstrate that the increase in deoxyribonucleic acid is not an obligatory consequence of the loss of chloroplasts in *Euglena*. That this augmentation is connected in some manner with the bleaching treatment is indicated by our findings with the bleached cells of type II and also by a recent report[7] describing a 50 per cent increase over the normal in the deoxyribonucleic acid contents of two bleached strains of *Euglena gracilis*. The reason for this phenomenon is obscure. It could, perhaps, be considered as significant that all bleached strains exhibiting the increase in deoxyribonucleic acid had been carried for several years before being analysed. Though the nature of the environmental stimulus giving rise to this alteration cannot be defined, a selection effect may be ruled out, since the cells characterized by a doubling of deoxyribonucleic acid, having a longer generation time, could scarcely have competed successfully, in the medium employed, with the organisms carrying the normal complement.

We are indebted to Dr. Arline D. Deitch for the examination of the preparations treated by the Feulgen procedure. This work was supported by research grants from the American Cancer Society and the National Science Foundation.

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Plastids of the *Avena* Coleoptile

Phototropic curvature of the *Avena* coleoptile may, for reasons which have been summarized elsewhere[1], be mediated through the plastids. Geotropic curvature may have a similar basis, for it has long been thought[2,3] that the geotropic responses of both shoots and roots are mediated by special starch grains or 'statoliths', and these in fact are plastids in which

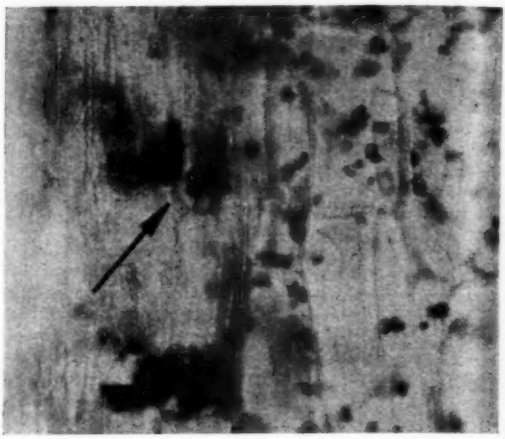

Fig. 1. From lower part of the coleoptile. Basal arrangement of the statoliths in two rows of cells only. (× 733)

relatively large amounts of starch have been deposited. It is thus worth considering that the many similarities between these two types of tropism may have an anatomical basis. For this reason a closer study has been made of the plastids of the etiolated *Avena* coleoptile. The observations have been made with the light and phase microscope, utilizing either fresh material vitally stained with neotetrazolium chloride, or microtome sections fixed in chrome-acetic-formalin and embedded and sectioned in 'Carbowax'. (We wish to thank Prof. R. H. Wetmore and Dr. J. Riopel for assistance and for putting at our disposal the newly developed 'Carbowax' method.) Principal attention has been directed to the most apical part of the coleoptile.

Plastids are seen in all cell-layers except the inner and outer epidermis, which appear not to contain them. Our observations indicate that they are of two types. The first do not react with neotetrazolium, when fully developed, and contain mainly starch, which stains red-brown with iodine. These occur in abundance at the very tip of the coleoptile and also in

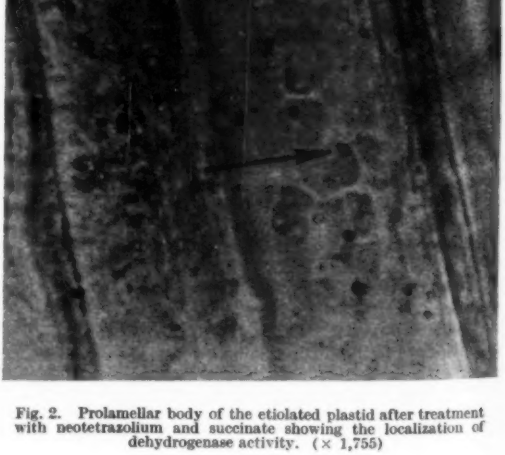

Fig. 2. Prolamellar body of the etiolated plastid after treatment with neotetrazolium and succinate showing the localization of dehydrogenase activity. (× 1,755)

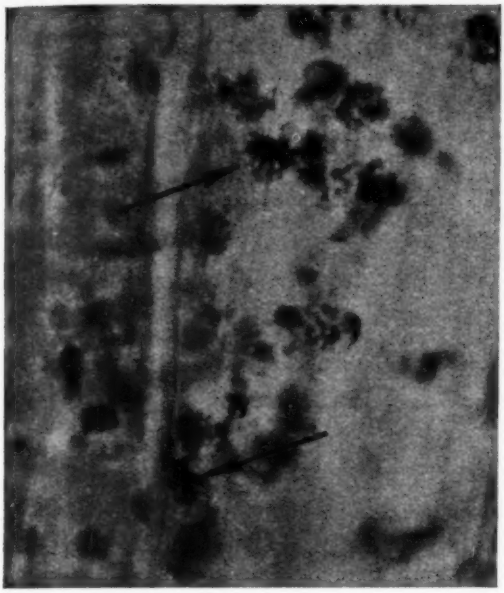

Fig. 3. Lobate outgrowth of the prolamellar body in cells similar to Fig. 2 after 1 hr. exposure to red light. Treatment as in Fig. 2. (× 1,755)

the subepidermal and middle layers around the fibro-vascular bundles. Thus these plastids are principally located in an arch rising outside and around the bundles; in the lower part of the coleoptile the sides of the arch have a thickness of two cells (Fig. 1), while the top has many layers. In plants which have been held vertical, sectioned under red light, mounted in iodine solution, and examined immediately, these bodies are found congregated at the basal end of the cells (Fig. 1). In plants which had been illuminated beforehand the basal arrangement of these plastids is not evident. Plastids of this type are without doubt identical with the statoliths described by von Guttenberg[4]; Yocum[5] has shown that these bodies roll down to rest on the lateral wall shortly after the plant has been placed horizontal. In the cells on the two sides remote from the bundles, these plastids occur sporadically and do not take up the characteristic basal position.

The second type occurs in highest concentration near the base of the tip portion, close to the apical termination of the vascular bundles; this point lies some 200μ below the extreme tip and 50μ above the uppermost end of the cavity. These plastics vary in size from 1 to 4μ and often contain a granule, 1–1·5μ in diameter, which stains strongly with neotetrazolium in presence of *M*/20 succinate or malate (Fig. 2). There is reason to identify this granule with the prolamellar body of etiolated plastids. Other cells contain these plastids in smaller numbers.

When the plants are illuminated with red light (wave-length 600 mμ and above) for 30 min. or more, the granule is found to have expanded into an *X* or lobate form (Fig. 3). This observation parallels that of the outgrowth of the 'crystalline' body into the lamellated structure of the typical chloroplast, which has been several times figured by the electron microscopists[6–9] and appears to establish this type of plastid as a pro-chloroplast. It is of interest, however, that the 'prolamellar body' is made evident by its dehydrogenase activity rather than by any special optical property. It is of still further interest that pre-exposure of the coleoptile to red light markedly modifies its subsequent phototropic sensitivity to white light[10]; such parallelism helps to support the concept of a close and causal relation between the proplastids and phototropic sensitivity.

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Anastomoses in the Phycomycetes

It is commonly assumed, either explicitly[1] or implicitly[2–4], that the vegetative mycelium of the Phycomycetes is incapable of vegetatively anastomosing and that this whole class of fungi is thereby precluded from developing complex fruiting-bodies and from undergoing variation by those means, such as heterokaryosis, which largely depend on the vegetative transfer of nuclei from the cells of one thallus to another.

Such an assumption is certainly confirmed by an examination of most cultures, but one of us (D. M. G.) has occasionally noticed the presence of anastomoses in a number of mycelia which, by their completely aseptate nature, are certainly those of Phycomycetes. Such cultures, isolated from soil in Britain and Australia, have usually been sterile, although one produced a few *Mortierella* sporangia, and the growth characteristics of the others lead us to believe that they belong to the same genus. Some are characterized by a normal system of scalariform anastomoses; but recently an isolate has come to hand in which the anastomoses are massive and comparatively crude, consisting of roughly conical projections, fused at their apices, from two adjacent hyphæ. In both types, extensive protoplasmic flow through mature anastomoses and their supporting hyphæ has been observed. A survey of other genera in the Phycomycetes, especially in the Aplanatae, and of other species of *Mortierella*, has failed to demonstrate the presence of further anastomoses, nor are they mentioned in Turner's[5] detailed description of *M. globulifera*.

This demonstration of the occurrence of anastomoses in at least one phycomycetous genus disrupts one of the "boundary conditions describing the Phycomycetes"[1]. It is also of interest in the fields of fungal variation and plant pathology, for it does not seem inconceivable that anastomoses could occur in other species under certain conditions, with the consequent possibility of heterokaryosis, etc. Such an occur-

rence would be particularly difficult to detect in the obligate pathogens of the Peronosporales.

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Occurrence and Function of the Provisional Embryonic Cuticle of a Lepidopteran

The provisional embryonic cuticle has been noticed in several groups of insects such as Aphidae, Orthoptera, Hemiptera, Neuroptera and some types of Coleoptera[1]. It was, therefore, of interest to note the presence of the provisional embryonic cuticle in the hitherto unreported group of Lepidoptera.

Various roles have been ascribed to the provisional embryonic cuticle. It has been described as acting as an "egg burster" by Wigglesworth and Sikes[2] and others. It is also regarded by some as affording protection to newly emerged nymphs of grasshoppers when the young ones crawl from the buried egg-pods to the surface.

I believe[3] that the moulting of the provisional embryonic cuticle in *Locustana pardalina*, *Tenebrio molitor* and *Dysdercus cingulatus* (unpublished work) is due to developmental causes. Moulting is induced in order to synchronize the rate of deposition of cuticle with the development of muscle ends, so that a proper integration between the muscle and the cuticular exoskeleton can take place. The provisional cuticle is precociously formed, in the sense that it is formed at a time when the muscles are not in a position to become attached to it by means of tonofibrils. While the other functional interpretations are certainly valid, there is reason for believing that they are secondary adaptations.

In *Attacus ricini* (Lepidoptera), the provisional embryonic cuticle was first detected histologically and also with the help of histochemical methods at about eighty hours before emergence. The second or definitive cuticle was secreted soon after the sloughing of the provisional embryonic cuticle. A thorough examination of the development of skeletal muscles showed that the muscle bands were in an early state of development, mostly in the form of myoblasts at the time of secretion of the provisional embryonic cuticle. The muscles were well formed at the time of the secretion of the definitive cuticle, and it is to this cuticle that the muscles could become properly attached (Fig. 1).

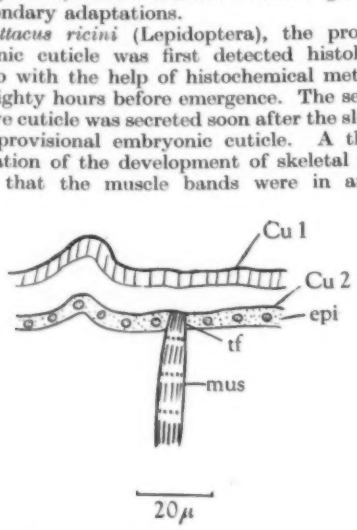

Fig. 1. epi., epidermis; Cu 1, provisional embryonic cuticle; Cu 2, definitive cuticle; mus., striated muscle fibre; tf., tonofibrils

The present investigation on *Attacus ricini*, therefore, confirms the conclusions reached for *Locustana*, *Tenebrio* and *Dysdercus*.

It is hoped to publish details elsewhere with Mr. Sahani of this Department. Mr. Barkat, of Government Farm, Ranchi, kindly made the eggs available for this work.

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Incorporation of Acetate into Protein by Obligately Parasitic and Saprophytic Fungi

The germ tubes of obligate parasites such as the rust fungi and powdery mildews fail to grow in the absence of host plants. This may be caused by metabolic blocks, the general location of which should be revealed by studies on the distribution of radioactive tracer among the various broad classes of metabolites. To determine if this would be a feasible approach to the problem of obligate parasitism, the assimilation of radiocarbon by uredospores of a rust fungus (*Uromyces phaseoli* (Pers.) Wint.) and conidia of a saprophyte (*Aspergillus niger* van Tiegh) was studied to see if any important differences in metabolism occurred between them. The preliminary experiments reported here were made with non-germinated spores, so the metabolism studied was essentially catabolic in character.

Spores of the two fungi were compared with respect to their capacities to incorporate the methyl carbon of $^{14}CH_3COONa$ into sugars, amino-acids, Krebs-cycle acids, proteins, nucleic acids, and lipids. Spores of each species were incubated with 10 μc. of $^{14}CH_3COONa$ for various time-periods and then killed with boiling 30 per cent ethanol. Techniques for the separation and analysis of the various components have been described elsewhere[1].

The results are given in Table 1 and Fig. 1. The principal differences between the two species occurred in the total activity incorporated and the patterns of

Table 1. Distribution of Carbon-14 in Amino-acids from Fungus Spores incubated 10 hr. with 10μc. $^{14}CH_3COONa$

Amino-acid	Free amino-acids		Protein amino-acids	
	U. phaseoli	*A. niger*	*U. phaseoli*	*A. niger*
Alanine	2	1	6	6
γ-Aminobutyrate	1	0	—	—
Arginine	2	0	3	1
Asparagine	2	0	—	—
Aspartate	13	4	29	23
Glutamate	20	46	43	45
Glutamine	40	28	—	—
Leucines	1	19	0	5
Lysine	1	0	11	11
Proline	—	—	1	3
Serine	1	0	5	3
Threonine	1	0	1	1
Tyrosine	0	0	0	0
Valine	13	0	0	1
Total c.p.m. per mgm. N	72,000	265,000	26,500	221,000

Results are given as percentage of total c.p.m. per mgm. protein nitrogen. Detectable label was not found in cysteine, methionine, phenylalanine, histidine or glycine. About 200 c.p.m. were required for detection by the methods employed.

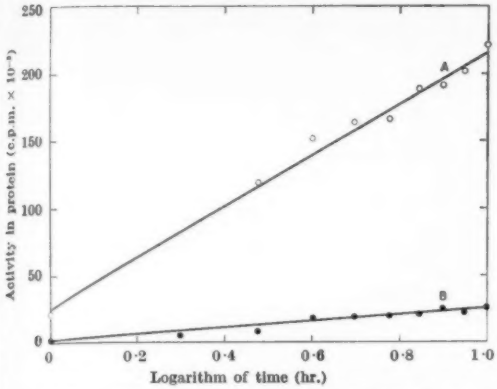

Fig. 1. Rate of incorporation of carbon-14 from $^{14}CH_3COONa$ into the protein fractions of: *A*, conidia of *A. niger*; *B*, uredospores of *U. phaseoli*

labelling of the protein fractions. Conidia of *A. niger* incorporated about 2,000,000 c.p.m. per mgm. of protein nitrogen during a 10-hr. exposure period, or about 10 times the amount of label assimilated by uredospores of the bean rust fungus. Most of the amino-acids in the protein fraction of *A. niger* became labelled in 10 hr. except for cysteine, methionine, phenylalanine, histidine, and glycine. Labelling in the free amino-acid fraction of this fungus was much more restricted, being confined to the leucines, glutamic acid, and glutamine. The specific activities of the amino-acids in the protein fraction of uredospores were much lower, and label was not found in valine or the leucines in addition to the acids not labelled by *A. niger*. A larger number of free amino-acids were labelled by bean rust spores than by *A. niger*; but specific activities were much lower than those isolated from conidia, reflecting low incorporation of exogenous substrate by uredospores.

Thus the rust spores were able to synthesize the same amino-acids from acetate as conidia of *A. niger*, but the over-all incorporation of substrate was lower. In addition, amino-acids were synthesized much more rapidly by conidia than by uredospores. For example, labelled valine was found in conidia in 2 hr., but did not appear in uredospores until 5–10 hr.

At least two amino-acids labelled by uredospores, valine, and leucine(s) were not incorporated into protein. This may have arisen from the fact that the capacity of uredospores for incorporating label into protein fell rapidly with time. When radioactivity in the protein fractions was plotted against the logarithm of time, linear relationships were obtained for both fungus species (Fig. 1). The regression equation relating carbon-14 assimilated, in terms of c.p.m. $\times 10^{-3}$ per mgm. protein nitrogen (L), to time of incubation in hr. (t) was found to be:

$$L = 26 \log_{10} t - 0{\cdot}8$$

for the bean rust fungus, with a correlation coefficient of +0·975.

For *A. niger* the regression equation was found to be:

$$L = 220 \log_{10} t + 3{\cdot}7$$

with a correlation coefficient of +0·995. Thus it is seen that the regression coefficient relating activity in the protein fraction to the logarithm of incubation time was 8·6 times higher for *A. niger* than for *U. phaseoli*, suggesting that failure of the latter species to incorporate valine and the leucines may arise from a loss of the mechanism for this incorporation.

Therefore, amino-acids which require long periods of time for synthesis may not become labelled soon enough for activity to be incorporated into protein. At least 5 hr. must elapse before the label can be detected in the free leucines isolated from rust spores, by which time incorporation of label into protein has fallen to a small fraction of the initial rate. The results indicate that the metabolism of resting uredospores is characterized by a rapid loss of ability to incorporate substrates into protein, coupled with low utilization of exogenous substrate, and a very slow rate of synthesis of some important amino-acids.

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MICROBIOLOGY

Adaptive Chain in the Conversion of Antimetabolites into Metabolites

THE adaptation of micro-organisms[1] and insects[2] to antimetabolites used in the control of the latter is an important contemporary problem. It has been conjectured that a possible mechanism for such adaptation might be: formation of new metabolic pathways, creation of inhibitors or enzymes neutralizing antimetabolites, or modification of the permeability of structures sensitive towards antimetabolites[3,4]. These mechanisms, in so far as they concern penicillin, would involve an induced biosynthesis of penicillinase which suppresses the antimetabolic activity of penicillin, an autotrophic biosynthesis of amino-acids the assimilation of which by the milieu is inhibited by penicillin[5], or a modification of bacterial surface structure[6]. All or any of these mechanisms might make the antimetabolite innocuous to the living cell.

The present investigation was undertaken to direct attention to some accessory mechanisms subsequent to the adaptation of micro-organisms to antimetabolites.

We established in 1956 (ref. 7) that, for penicillin-resistant *Staphylococci* (not penicillin-dependent), benzylpenicillin serves as a growth factor, replacing thiamine in a nutrient medium containing nicotinic acid[8] but deficient in thiamine. We found that this effect occurs solely with benzylpenicillin and its derivatives, which lack antibacterial activity, benzylpenicilloinic and benzylpenillic acids; it does not occur with other derivatives of penicillin and similar antibiotics[9]. The growth effect observed for concentrations of 10^{-10}–10^{-11} M is of the same order as that due to thiamine. A similar growth effect due to vitamins was observed only in the case of thiamine pyrophosphate, thiamine and biotin.

These results should show whether, as a result of adaptation to benzylpenicillin, there appears a novel growth factor, or whether it is converted to thiamine

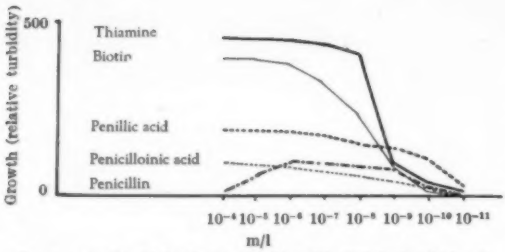

Fig. 1. Growth of *Staph. aureus* 671, cultivated during 48 hr. at 37° C. on a nutrient medium containing $1{\cdot}6 \times 10^{-6}$ *M* nicotinic acid (ref. 8), as well as the substances named at concentrations given on the abscissa

or biotin. In order to clarify the mechanism involved, the effect of the substances named in Fig. 1 on various metabolic processes has been studied.

With *Staphylococci* deficient in thiamine and resistant to penicillin[10], it has been established that thiamine, penicillin and biotin all activate respiration in a similar degree, approximately 60 per cent, on a glucose substrate. Respiration in the case of pyruvate and lactate as substrates is activated particularly by thiamine and thiamine-pyrophosphate, and rather less—though to a practically similar degree—by biotin and benzylpenicillin (also benzylpenicilloinic and benzylpenillic acids); see Table 1.

Table 1. Respiration of *Staph. aureus* with 0·025 *M* Pyruvate or Lactate as Substrate and Concentration of Substances tested $2{\cdot}5 \times 10^{-5}$ *M*

	Thiamine	Biotin	Penicillin	Control
		Substrate pyruvate		
Q_{O_2}	7·84	6·12	5·67	4·90
Per cent	160	125	116	100
		Substrate lactate		
Q_{O_2}	34·3	26·6	29·2	21·8
Per cent	157	122	134	100

Penicillin stimulates the respiration of penicillin-resistant *Staphylococci*. Thus, if our penicillin-resistant strain (*Staph. aureus* 315) is cultivated on a nutrient medium containing benzylpenicillin in a concentration equal to that showing resistance, but not with the object of modifying resistance, the activating effect of the penicillin on respiration is increased about threefold as compared to the initial stage. The stimulating effect of penicillin on respiration is not shown immediately on the introduction of penicillin, but after some 45 min.; whereas with thiamine, thiamine-pyrophosphate and biotin, activation of respiration is shown immediately on their introduction.

These tests suggest that penicillin is an adaptively formative growth factor, probably different from thiamine and related to or identical with biotin.

In order to study the interaction between penicillin and biotin as metabolites for penicillin-resistant *Staphylococci*, we have tested the introduction of the carbon from bicarbonate into the protein mass of penicillin-resistant *Staphylococci* (Table 2).

Conditions were as follows: 2 hr. incubation at 40° C. in a medium containing, in ml.: 4 mgm. casein hydrolysate-vitamin free; 5 mgm. dipotassium hydrogen phosphate, 4 mgm. glucose, 0·8 μc. sodium bicarbonate-^{14}C, nicotinic acid, 10^{-9} *M*, substances under test at 10^{-9} *M* concentration, micro-organisms 3·4–4·6 mgm. nitrogen and *p*H 7·4. The results are given in impulses/min. for 0·6 mgm. protein nitrogen, while the values in brackets indicate the percentage relating to the control less growth-factor. The tests were carried out with the penicillin-sensitive strain 209, its resistant variant 209*P*, obtained by culture on nutrient media containing penicillin and the penicillin-resistant strain 671.

Likewise, we have measured the rate of introduction of methionine-^{35}S. Under similar conditions, the increased rate of introduction of amino-acid under the influence of penicillin was found not to exceed 10 per cent.

The results obtained indicate that penicillin, similar to biotin, activates the fixation of carbon dioxide (Wood and Werkman reaction), forming products presumably converted into amino-acids, utilized for protein synthesis. It is difficult to appreciate whether the penicillin is adaptively converted into biotin, or whether it acts as a new, adaptively formed metabolite synergistic to biotin. With resistant strains, the rate of introduction of carbon dioxide into the protein mass is many times greater than in the sensitive strains. This is probably the manifestation of the autotrophic formation established by Gale and Rodwell[5] of the amino-acids necessary for protein synthesis in the case of penicillin-resistant *Staphylococci*. Evidently, penicillin as a metabolite for penicillin-resistant *Staphylococci* is introduced precisely into those links of the metabolism which develop with the establishment of resistance.

Our results show that, in the case of penicillin-resistant *Staphylococci*, both penicillin and its product for rendering penicillinase innocuous—penicilloinic acid—serve as metabolites. Similar cases have been described with other metabolites. Thus, chloramphenicol is made innocuous by bacteria, being transformed into *p*-aminobenzoic acid[11]. The anti-metabolite of riboflavin-6-chloro-7-methyl-9-(1′-D-ribityl)-*iso*alcosasin may be adaptively utilized by *Lactobacillus casei* as a metabolite[12].

These manifestations lead us to believe that they are an expression of a more general biological occurrence of an adaptive conversion of antimetabolites to metabolites. The chain of complete adaptation as regards some metabolites may be rendered schematically thus:

antimetabolite → indifferent product → metabolite

The adaptation of *Staphylococci* to penicillin observed by us embraces this entire, complete adaptive process. Further, the penicillin antimetabolite converted into a metabolite is launched into a diametrically contrary direction, assisting the organ-

Table 2. Introduction of the Carbon from Bicarbonate into the Protein of *Staphylococci* under the Influence of Various Factors

Strain	Resistance (*E.*/ml.)	Control	Biotin	Penicillin	Thiamine
Staph. aureus 209	0·03	218 (100)	288 (132)	147 (68)	257 (118)
Staph. aureus 209*P*	5,000	297 (100)	365 (123)	344 (116)	293 (99)
Staph. aureus 671	1,000	839 (100)	1,128 (134)	1,006 (120)	727 (87)

ization of autotrophic mechanisms which lead to the by-passing of metabolic links affected by penicillin.

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A Vacuolar System in Cells of *Agrobacterium tumefaciens* as shown by Electron Microscopy of Ultra-thin Sections

Electron microscope studies of ultra-thin sections of the cells of several species of bacteria made by different workers have shown a regular structural pattern which can be summarized as follows: a definite cell wall, a cytoplasmic membrane (Chapman[1]), a granular cytoplasm with one or more round, dense inclusions called chondrioids (Kellenberger *et al.*[2]), and a more or less central core which is irregular in shape and low in density to the electron beam. Comparative studies of stained preparations with the light microscope have led various workers to associate this core with nuclear structures, and it has been called vesicular nucleus, kernvacuole, kernäquivalent, nucleoide, nuclear sites, and nuclear apparatus. Reticulated structures within this core have been considered to be nuclear material, deoxyribonucleic acid or even chromosomes.

Cells of *Agrobacterium tumefaciens* grown on nutrient agar slants for 48 hr. were fixed in potassium permanganate, incorporated into agar blocks, and embedded in methacrylate. Ultra-thin sections were cut with a diamond knife and photographed in the R.C.A. *EMU3B* electron microscope. In the micrographs we have obtained, we have observed some of the structures described above (Fig. 1). However, after removal of the methacrylate with benzene, followed by shadow-casting with palladium, the central core of low electron density is observed as a vacuole crossed by a few strands and with some dense granules protruding from the cytoplasm into it (Fig. 2). Structures that could represent a vacuolar membrane have not been observed.

The presence of a vacuolar system had been observed in other bacteria: Houwink[3] showed in *Halobacterium halobium* a complex vacuolar system not related with the nucleus; and Knaysi[4] working

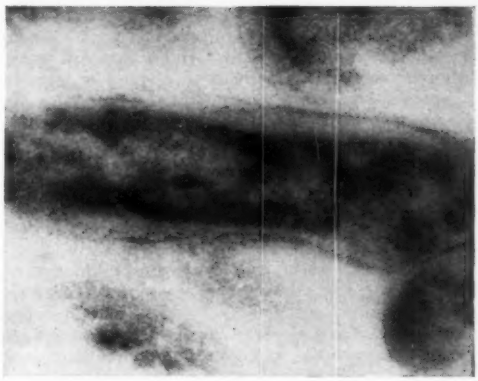

Fig. 1. Cells fixed in 5 per cent potassium permanganate in veronal–acetate buffer, *p*H 7·5, embedded in methacrylate. (× 42,000)

with *Mycobacterium thamnopheos* stated that "the destructive effect of acid hydrolysis . . . is attributed to the presence of vacuoles in the cells of this organism". The empty vacuoles revealed in our work do not seem to correspond to regions with appreciable concentration of deoxyribonucleic acid. Also the absence of a definite membrane limiting these vacuoles, as Birch-Andersen and Maaløe[5] pointed out, gives evidence that the vacuoles with their contents are structures not related with distinct nuclei which are generally separated from the rest of the cell, but probably correspond to the vacuolar systems found in many other vegetative cells.

The work of Knaysi[6] on *Bacillus cereus*, in which he observed the division and fusion of dense granules, indicates that the nuclear sites correspond to areas denser than the cytoplasm, and that some of the so-called cytoplasmic inclusions could be the real sites of the nuclei. Preusser[7], studying ultra-thin sections of *Escherichia coli*, stated that the zones of low electron density within the bacterial cells are products of cellular metabolism and are not comparable with the nuclei of higher organisms. On the other hand, the correspondence observed between the light areas in electron micrographs of ultra-thin sections and the stained nuclear material observed in the light microscope is probably due to the fact that the whole thickness of the bacterial cell is in focus in the light

Fig. 2. Cells fixed, embedded and sectioned as in Fig. 1, methacrylate removed with benzene and section shadow-cast with palladium. (× 34,700)

microscope, having been flattened by fixation or drying. It is, therefore, impossible to be certain whether the stained matter which appears within the lighter areas is actually within them or is within the cytoplasm above or below the vacuoles and appears superimposed on them. In any event, the vacuoles observed as clear areas in ultra-thin sections do not correspond to any of the nuclear systems we know at present. In micro-organisms where the location of the real nucleus is known (for example, yeast cells) one notes that the nuclei are not in vacuoles but are embedded in the cytoplasm.

Although only 48-hr. cultures were included in the present work, and although there may be variation between species, we are of the opinion that the central core or zone of low electron density in *A. tumefaciens* represents a vacuolar system and is probably not the site of the bacterial nucleus or nuclear equivalent.

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Capacity of Extracts from Prestarved *Escherichia coli* to incorporate Thymidine into Deoxyribonucleic Acid

The ability of a polyauxotroph, *Escherichia coli* strain 15_T- (555–7), to synthesize deoxyribonucleic acid is temporarily lost following a period of incubation in a minimal salts–glucose medium containing thymine but no added amino-acids[1–4]. Moreover, when the starved cells are returned to a medium supplemented with amino-acid, a lag in synthesis of deoxyribonucleic acid is observed[2–4]. The restoration of an active deoxyribonucleic acid synthesizing system in these cells can be prevented by continued suppression of protein synthesis with chloramphenicol[3] or by exposure to X-rays[5]. One possible mechanism considered was that the enzyme or enzymes involved in deoxyribonucleic acid synthesis are lost or destroyed during the starvation period and that chloramphenicol and X-ray exposure prevent resynthesis or reactivation of these components[2–5].

In a study of incorporation of thymidine labelled with tritium into deoxyribonucleic acid I have determined that polymerase and the kinases[6] responsible for phosphorylation of thymidine are present in the starved cell.

The extracts tested were obtained from cells broken in a French pressure cell. Intact cells and debris were pelleted by centrifugation at 10,000*g* for 20 min. Less than 10 colony-forming cells per mgm. protein were found in the extracts so obtained. The procedure for studying thymidine incorporation into deoxyribonucleic acid was a modified version of that used by Kornberg and collaborators[7,8]. Experiments 1 and 2 were conducted with batches of cells obtained from independent inocula. In the third experiment half an actively growing log-phase culture was used for preparation of the log-phase cell extract and the remaining portion put into medium deficient in amino-acid following harvesting and washing for preparation of the appropriate starved cell extract. Thus, in the latter experiment both cell suspensions were derived from the same culture batch. As can be seen in Table 1, the multi-enzyme system needed for thymidine incorporation was present in extracts obtained either from log-phase cells or cells which had been incubated for 90 min. at 37° C. in minimal medium supplemented with thymine but lacking the required amino-acids arginine, tryptophan and methionine ('starved cells'). These results are in contrast to the impaired *in vivo* deoxyribonucleic acid synthesizing capacity of the starved cells. I am investigating the possibility that thymidine incorporation in the absence of net deoxyribonucleic acid synthesis may occur *in vivo*.

Table 1. Incorporation of Thymidine labelled with Tritium into Deoxyribonucleic Acid

Experiment	Conditions	mμmoles ^{3}H-thymidine incorporated into deoxyribonucleic acid/mgm. protein*
(1) Extract from log phase cells	Complete system†	1·21
	Heated extract	0·01
	Minus adenosine triphosphate and phosphoglyceric acid	0·01
	Minus thymus deoxyribonucleic acid	1·02
(2) Extract from starved cells	Complete system	1·45
	Minus thymus deoxyribonucleic acid	1·35
(3*A*) Extract from log phase cells	Complete system	2·00
	Minus adenosine triphosphate and phosphoglyceric acid	0·02
	Minus the triphosphates of deoxyadenosine, deoxycytidine and deoxyguanosine	0·15
(3*B*) Extract from starved cells	Complete system	1·75
	Minus the triphosphates of deoxyadenosine, deoxycytidine and deoxyguanosine	0·20

* After incubation at 37° C. for 20 min., the reaction vessels were placed in ice and 0·2 ml. of carrier deoxyribonucleic acid (2·5 mgm./ml.) was added. The immediate addition of 0·5 ml. of cold 10 per cent perchloric acid stopped the reaction and precipitated the cold-acid insoluble material. After 10 min. the precipitate was broken up with a glass pestle and washed with a total of 2 ml. of cold distilled water. The precipitate was then centrifuged and the pellet dissolved in 0·2 *N* sodium hydroxide. The solution was reprecipitated with cold perchloric acid. This was repeated twice. The final precipitate was hydrolysed in 1 ml. of 5 per cent trichloracetic acid for 30 min. at 100° C. The hot-acid soluble supernatant was then assayed for radioactivity.

† The complete incubation mixtures (0·30 ml.) contained: 0·02 ml. of glycine buffer (1 *M*, *p*H 9·2); 0·02 ml. magnesium chloride (0·1 *M*); 0·03 ml. of 2-mercaptoethanol (0·01 *M*); 0·02 ml. each of the triphosphates of deoxyadenosine, deoxyguanosine and deoxycytosine (500 mμmoles/ml.); 0·02 ml. of thymus deoxyribonucleic acid (Sigma Corp.) that had been previously heated at 100° C. for 5 min. (0·5 mgm./ml.); 0·01 ml. of adenosine triphosphate (100 μmoles/ml.); 0·01 ml. of phosphoglyceric acid (0·05 *M*); 0·02 ml. of labelled thymidine (262 mμmoles/ml., $1·54 \times 10^8$ counts per min. per mμmole on the Packard liquid scintillation counter); extract containing 685 μgm., 535 μgm., 253 μgm., and 282 μgm. protein was added from experiments 1, 2, 3*A* and 3*B* respectively.

That the thymidine was being incorporated into the deoxyribonucleic acid by the multi-enzyme preparation from starved cells was shown by the observation that all the label was associated with the alkali-resistant portion of the cold-acid insoluble material. It is possible that formation of one or more of the necessary deoxyribonucleotides may be blocked in the starved cells. Gros and Gros working with other strains of *E. coli* requiring amino-acid reported the unimpaired capacity of amino-acid starved cells to form mononucleotides[1]. Whether this is true for the deoxyribotides remains to be determined. However, if the presence of thymidine phosphorylating enzymes is indicative of the presence of all enzymes involved in forming the triphosphorylated deoxyriboside substrates necessary for deoxyribonucleic acid synthesis, then I must look elsewhere to explain the discrepancy between the *in vivo* and *in vitro* studies. The possibility of an accumulation of inhibitors during amino-acid deficient growth must be considered. Perhaps there is an unstable protein needed *in vivo* to convert non-priming deoxyribonucleic acid into an active primer. In this work the extracts from both normal and starved cells were found to contain deoxyribonucleic acid capable of serving as primer (Table 1). This may not reflect the true *in vivo* situation since deoxyribonucleases and possibly other enzymes may be activated following rupture of the cell[7,9].

This work is continuing and will be published in more detail elsewhere.

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Effect of the Peritoneal Fluid of the Guinea Pig on Strains of Enteric Bacilli

ALTHOUGH the complement-dependent bactericidal system of normal mammalian serum has been actively studied since the early descriptions of Nuttall[1] and Buchner[2], its role in native immunity is a matter of disagreement[3]. Recent reports indicate that strains of Gram-negative enteric bacilli isolated from patients' blood or from burns are ordinarily resistant to this system[4,5]. When equal numbers of a strain of *E. coli* resistant to this bactericidal effect and of one sensitive to it are injected into a rabbit, both strains ordinarily can be cultured from the blood stream 24 hr. following injection; but organisms from the resistant strain are 10–20 times more numerous[6]. There have been reports that strains of enteric bacilli resistant to the action of the complement-dependent system are more virulent for the mouse than are sensitive strains[7,8]. Such observations lead to the belief that bacterial strains sensitive to the action of this system are less likely to cause or maintain an infection than are resistant strains.

During the work on the bactericidal properties of human serum[4], it was noted that pleural or peritoneal transudates from patients with heart failure were active against strains sensitive to serum. This activity was considerably less than that of serum and could be destroyed by heating the transudates at 56° for 30 min.

One method of assessing the role of the complement-dependent system of peritoneal fluid appeared to be the intraperitoneal implantation of chambers containing sensitive and resistant bacterial strains. These chambers were designed to be permeable to body fluids but not to bacterial or mammalian cells[9]. The degree of sensitivity of strains to normal guinea pig and human sera was measured initially by determining the decrease in viable count following exposure of known numbers of bacteria to undiluted serum for 2 hr. at 37°. None of these bacterial strains was killed by heat-inactivated serum. The thirteen strains of enteric bacilli listed in Table 1 were used. Two sensitive strains and two resistant strains were ordinarily used as inocula in the four chambers inserted in each guinea pig.

Table 1. THE RELATIONSHIP OF THE SENSITIVITY OF BACILLARY STRAINS TO THE BACTERICIDAL EFFECT OF NORMAL SERA AND THEIR SURVIVAL IN INTRAPERITONEAL CAPSULES

Bacterial strain		No. bacteria killed/ml. serum in 2 hr.		Growth in intraperitoneal chambers
		Guinea pig	Human	No. times grew/No. trials
Klebsiella	15	160×10^6	160×10^6	0/5
Shigella	141	110×10^6	110×10^6	0/4
E. coli:				
	*A*9	170×10^6	170×10^5	0/4
	128	240×10^4	240×10^6	1/4
	*A*14	170×10^4	170×10^6	0/3
	182	168×10^2	168×10^4	4/14
	31	200×10^2	200×10^3	6/12
	*A*30	1,500	2,100	1/2
	108	140	0	14/15
	*A*13	98	143	3/5
	114	95	0	3/3
	102	54	0	12/15
	*A*39	19	0	3/3

Drum-shaped chambers about 6 mm. deep and 13 mm. in diameter were constructed from lengths of 'Tygon' tubing, the ends of which were covered with 'Millipore' membranes having a pore diameter of 0·45μ (ref. 9). 'Plexiglass' dissolved in ethylene dichloride was used as adhesive. The 'Millipore' membranes were autoclaved and the tubing boiled for 5 min. before the membranes were attached aseptically. Two No. 26 hypodermic needles were inserted through the wall of each chamber. One served as an outlet for air. Usually about 1,000 organisms suspended in physiological saline were injected through the other. The needle holes were sealed with a heated glass rod. Inocula up to 14,000 organisms were used in some experiments; but in any given experiment the numbers were approximately equal. Four of the chambers were then inserted into the peritoneal cavity of a guinea pig. These were removed at 48 or 72 hr. and the number of bacteria in each chamber determined by viable count. Sterility check on the membranes of the chambers at insertion and on removal showed no contamination, and no leakage.

Fluid from the chambers was examined by paper electrophoresis and the major serum fractions were present 24 hr. after the insertion. At 48 hr. the concentration of these fractions as estimated by their electrophoretic curves was about 60 per cent of that of guinea pig serum. Specimens from 40 chambers were examined by phase microscopy, and no mammalian cells were seen.

In every instance except two, the contents of the chambers either were bacteriologically sterile or showed a multiplication of the original inoculum to 1×10^6 to 1×10^9 organisms per chamber. In the two exceptions, the count had been reduced to less than fifty and these two instances are considered in the same category as the sterile ones

Table 1 lists the strains in their order of sensitivity to guinea pig serum *in vitro*. The numbers represent the maximum number of each strain killed by 1 ml. of undiluted serum. Close relationship between sensitivity *in vitro* to human serum and to guinea pig serum is apparent. A similar relationship between sensitivity to human serum and rabbit serum has been shown[6] and leads one to believe that results from experiments in one species may have implications for other species.

The column on the right in Table 1 shows the fate of the strains in the chambers. The very sensitive strains were killed whereas the most resistant strains usually multiplied. Strains of intermediate sensitivity multiplied in some instances but were killed in others. If a strain of intermediate sensitivity multiplied, it did so to the same extent as did resistant strains in the same animal.

These data serve to strengthen the belief that the complement-dependent bactericidal system is of importance in limiting the number of bacterial strains capable of surviving in tissue fluids *in vivo*.

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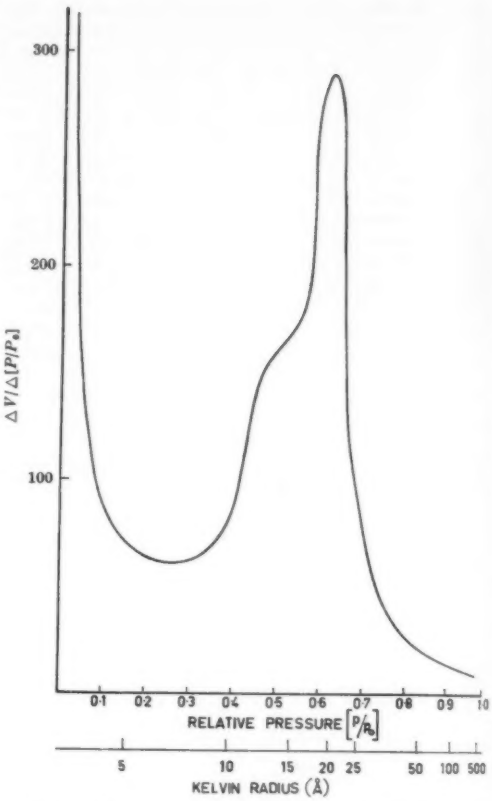

Fig. 1. Pore size distribution of calcium Willalooka illite. Because the clay crystals are plate-shaped the Kelvin cylindrical radii can be interpreted as crystal separations

An illite clay from the hundred of Willalooka in South Australia was saturated with sodium and calcium by repeated washing with a molar solution of the appropriate cation. The clay was dialysed against distilled water, dried and ground to powder which was then allowed to come to equilibrium with a relative vapour pressure of 0·96 before being compressed into cores[2]. These cores were wet up to pF 2 and dried in stages to complete desiccation over phosphorus pentoxide. Water and nitrogen isotherms were obtained using these cores. This illite, although low in potassium (potassium oxide, 4 per cent), gives no evidence of interstratification.

A complete adsorption–desorption isotherm of nitrogen was obtained at 78° K. The surface area of the clay calculated by the B.E.T. method was 150 m.²/gm. In Fig. 1 a plot of $dV/d(p/p_0)$ against p/p_0 is shown together with a scale to relate values of p/p_0 to the Kelvin cylindrical pore radius. The clay cores have a porosity of 18·8 c.c./100 gm. and at $p/p_0 = 0{\cdot}80$, 18·6 c.c./100 gm. of pores are filled, indicating that almost the entire porosity is contained in pores less than 42 Å. Kelvin radius. Since the plates of this mineral are 700 Å. across and this is large compared with the plate separation, the calculated Kelvin cylindrical radius can more properly be regarded as a measure of the plate separation. The pore peak in Fig. 1 shows that the clay mass can be regarded as a series of parallel crystals, the

SOIL SCIENCE

Domain or Turbostratic Structure of Clays

It has been realized for some time that plate-shaped clay particles on drying tend to take up a parallel orientation with respect to one another. Further, Terzaghi[1] has indicated that groups of parallel particles behave as a single particle in suspension and has referred to these groups as 'clay clusters'. Aylmore and Quirk[2], in endeavouring to interpret the swelling of clay-water systems, have defined a 'domain' as a parallel arrangement of crystals. Coal research workers[3] have referred to a similar type of organization as 'turbostratic groups' since the elementary particles are arranged in a parallel fashion in groups and these groups are arranged in turbulent array. The present communication presents evidence for the nature of a domain and discusses the consequence of domain formation in swelling phenomena of clays.

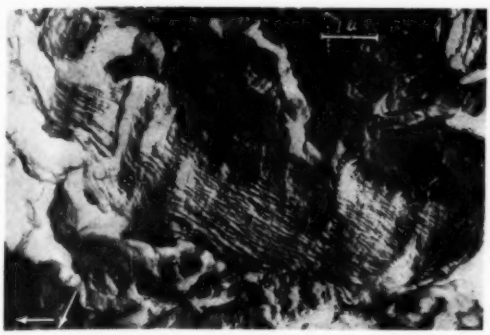

Fig. 2. Electron micrograph of the fracture surface of Willalooka illite core obtained using a replica technique. Arrows indicate the direction of uranium shadowing

separation of which varies between 10 and 30 Å. although closer approach may occur at a few points. This indicates that the pore space of this material is almost exclusively within domains, that is, there is very little interdomain porosity. An electron micrograph of a shadowed replica of the fracture surface of a dry clay core reveals the parallel arrangement of the clay particles and also indicates that there is considerable distortion of the crystals and domain structures undoubtedly caused by stresses set up in drying (Fig. 2).

In Figs. 3 and 4 the solution content–energy curves for sodium and calcium Willalooka illite at varying sodium chloride and calcium chloride concentrations are shown. Since the clay is largely organized on a domain basis it appears permissible to divide the solution content by the specific surface area to obtain a first approximation for the film thickness on the clay surface. When this is done, good agreement between Schofield's theory based on the Gouy–Chapman diffuse double layer and the experimental values for sodium-illite are found for 10^{-1}, 10^{-2} and 10^{-4} M sodium chloride concentration.

The remarkable insensitivity of the swelling of the calcium illite to calcium chloride concentration and the appreciable hysteresis in the water content suction curves indicates the non-applicability of diffuse double layer theory to the swelling of this material.

The explanation of the film thickness at pF 1 of 30 Å. even for distilled water and the presence of hysteresis lies in the existence of a potential barrier at a plate separation of < 60 Å. and the formation of a gel structure as the result of the relaxation of strains when sufficient liquid is present in the clay matrix. The limited swelling cannot be ascribed to cementing materials; when the sodium illite, swollen in distilled water, is converted to a calcium illite and dried, identical swelling behaviour to the calcium illite prepared directly from the natural material is observed. MacEwan[4] has suggested that coulombic attractive forces exist up to plate separations of 30 Å., at which separation he envisaged the formation of separate double layers by each particle. It seems reasonable to conclude that calcium illite does not overcome this barrier, and that for sodium illite the barrier is overcome, giving rise to high solution contents which are dependent on the sodium chloride concentration. The similarity of this behaviour to that of sodium and calcium montmorillonite[5] is striking and supports the suggestion that a domain structure in some respects resembles a montmorillonite crystal. It is also important to note that on heating to 400° C. the sodium illite swells very little but still retains its exchange capacity of 0·41 m.equiv./gm.

One difficulty in interpreting the water content of a clay in terms of the film thickness developed by the clay particle is that, on wetting, relaxation of strains[6] leads to the formation of a gel structure so that it is difficult to separate that water which is

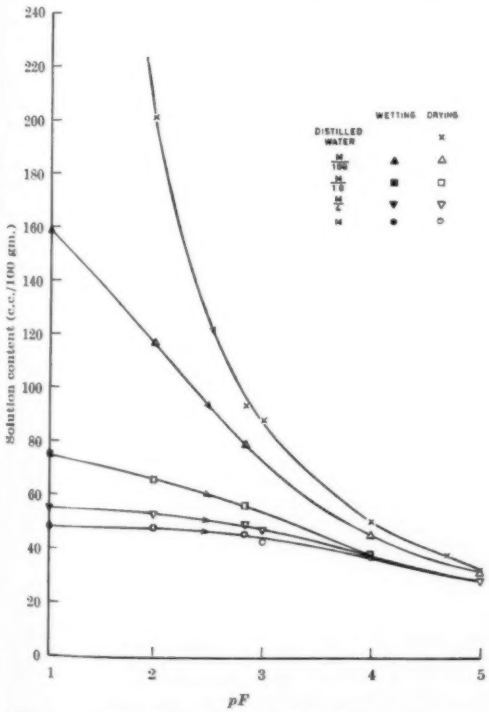

Fig. 3. Solution content–energy curves for sodium Willalooka illite at various electrolyte concentrations (sodium chloride). Note that pF indicates only the logarithm of the hydrostatic suction in cm. of water

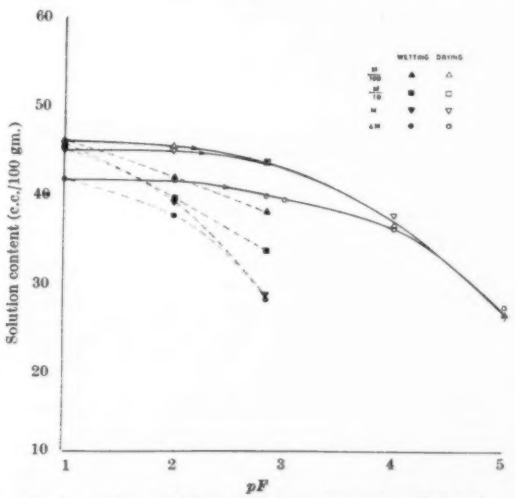

Fig. 4. Solution content–energy curve for calcium Wallalooka illite at various electrolyte concentrations (calcium chloride)

directly associated with the clay surfaces from water which is simply enmeshed within the gel structure. Enmeshed water can be regarded in the following way. When a rigid porous material initially filled with liquid dries out under a falling vapour pressure, pores (capillaries) of particular dimensions will empty at a particular vapour pressure. The remaining liquid is held under a hydrostatic suction which is transmitted to the walls but, as each pore empties, stresses on the walls due to this cause will fall to zero. If, however, the material is easily deformed, the suction in the liquid will cause a reduction in the size of the pore, and evaporation will be delayed until a lower vapour pressure is reached, by which time the suction has further increased. The mechanism has been postulated by Barkas[7] for wood-gels, but is clearly applicable to the interpretation of gel structures and hystereses in clay–water systems.

In conclusion, it is necessary to comment on two recent papers which have overlooked, wholly or in part, suggestions put forward at an earlier date by us. Greacen[8], in discussing the swelling of calcium montmorillonite in terms of the critical voids ratio line, has used a surface area of 800 m.²/gm. and has not adequately taken into account the presence of a gel structure. Blackmore and Warkentin[9] have also claimed that their results support the existence of diffuse double layers on the external surface of montmorillonite crystals. However, they too have not considered the possibility that much of the water retained by their clay was enmeshed in a gel structure. Further, Norrish[10] and also Blackmore and Warkentin[9] have noted a collapse of swollen crystals or a condensation of elementary silicate sheets of sodium montmorillonite to give a 19 Å. spacing in the presence of dilute calcium chloride solutions. This situation indicates that attractive forces in calcium clay systems must be such that diffuse double layers do not readily form.

The results discussed here, and those obtained for kaolinites and montmorillonites which will be presented elsewhere[11,12], indicate that diffuse double-layer formation is not of any great significance for the swelling of calcium clays. Diffuse double layers may exist for crystals of calcium clays in a suspension, but this is doubtful in view of the negative adsorption results obtained for an illite[13].

We are indebted to Mr. D. M. Hall, of the Department of Scientific and Industrial Research Dominion Physical Laboratory, for obtaining the electron micrograph.

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VIROLOGY

Polyphenol Oxidase Activity of *Nicotiana glutinosa* Leaves infected with Tobacco Mosaic Virus

When local necrotic lesions appeared on the leaves of *Nicotiana glutinosa* L. infected with tobacco mosaic virus, but not before, the respiration-rate increased[1]. The same phenomenon was observed on some other host–virus combinations which produced local necrotic lesions[2]. In these cases, it seems that the increased rate of respiration varies with various developmental stages of the necrotic lesions, namely, with degrees of brownish discoloration of leaf tissue due to the virus infection. When the tissues of the tobacco plant are injured, the polyphenol substances are oxidized and then polymerized by polyphenol oxidase[3]. Therefore the polyphenol oxidase activity of leaves bearing necrotic lesions has been compared with that of healthy leaves.

The half-leaves of *N. glutinosa* plants were inoculated mechanically with tobacco mosaic virus and healthy control halves were treated with healthy tobacco leaf sap. Local lesions became visible the second day after inoculation at 20° C. Leaves were harvested on the day following inoculation; when no lesions appeared, the second and the third day after inoculation. Polyphenol oxidase activity was measured in crude enzyme preparations: acetone powder prepared from healthy and infected half-leaves, by the use of Warburg respirometer. The results are expressed by μl. oxygen absorbed by acetone powder (30 mgm. dissolving in solution containing 0·05 *M* phosphate buffer, *p*H 7·0, and 0·2 *M* sucrose) on addition of catechol (0·05 *M*) as a substrate at the rate of once an hour and once every 2 hr. in the case of gallic acid (0·05 *M*). These values have been corrected for autoxidation values and represent the mean value of several experiments. Oxygen consumption in each experiment varied so much according to the leaves chosen or preparative procedure of acetone powder that the standard error of the difference between the values for healthy and infected leaves was calculated (Table 1).

Table 1. Polyphenol Oxidase Activity of *N. glutinosa* Half-leaves inoculated with Tobacco Mosaic Virus and of Healthy Halves

Days after inoculation	Polyphenol oxidase activity: Infected	Healthy	Ratio Inf. : Healthy	*S.E.*	No. of determinations
Catechol substrate					
1	31·8	31·2	1·02	±1·44	5
2	51·7	38·5	1·44	±1·29	6
3	70·4	43·9	1·60	±6·69	6
Gallic acid substrate					
1	12·1	15·3	0·79	±3·18	5
2	17·5	14·5	1·21	±2·89	6
3	27·1	12·6	2·15	±3·66	6

As shown in Table 1, a considerable increase in activity of polyphenol oxidase was found the third day after inoculation by using either catechol or gallic acid as substrate. In contrast, there was no increase in the activity on the day following inoculation, having no visible lesions, on either substrate. On the second day, when faint black spots had become visible, the ratio of the activity between infected and healthy was 1·44 by using catechol

as substrate, and its difference was highly significant, while the difference is not significant at the 5 per cent level in the case of gallic acid although the ratio is 1·21.

In spite of much research, present knowledge of the precise nature of phenol oxidase is limited, and its role in cellular respiration is not as yet clear[4]. It seems, however, that the enhanced activity of polyphenol oxidase plays an important part in browning of plant tissue or hypersensitive defence reaction in both fungal and viral infection[5]. It is unknown whether or not the polyphenol oxidase is a catalyst mediating the final reaction with oxygen in cellular respiration of *N. glutinosa* leaves. The findings, however, that no increase in the activity is detected on the following day after inoculation and the increased rate of the third day is higher than that of the second day were apparently consistent with the results obtained by Yamaguchi and Hirai in the respiration measurement of *N. glutinosa* leaf disks infected with the same virus[1]. Recently, Solymosy *et al.*[5] offered clear-cut evidence that the stimulated activity of phenol oxidase was the immediate cause of lesion formation, by using the technique of administration of compounds capable of reducing quinones or inhibiting lesion formation. This seems to explain the biochemical mechanism of lesion formation. We cannot explain, however, the cause of the parallelism between increased oxygen consumption in infected leaf disks and increased polyphenol oxidase activity during lesion development. This raises the problem of the nature of terminal oxidase in infected tissues.

We are indebted to Prof. Tokuzo Hirai for valuable discussions.

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Direct Infra-Red Spectral Analysis of the Cucumber Mosaic Virus Infection Process

Infra-red spectroscopy may provide a means of following biochemical changes which occur in tissues as a result of virus infection, if induced spectral changes can be detected and related to the structure of the absorbing compounds.

The upper surfaces of fully expanded cotyledons of cucumber seedlings were dusted with 'Carborundum' and inoculated with a small sponge rubber pad moistened with tissue homogenates of tobacco plants infected with cucumber mosaic virus. Identically grown control plants in separate pots were dusted with 'Carborundum' and rubbed with virus-free buffer in the same manner. Control and virus-inoculated plants were held in a growth chamber with a 16-hr. day at 28° C. and an 8-hr. night at 20° C.

Lower epidermis was stripped from three infected and from three normal plants at daily intervals following inoculation. Adhesive 'Cellophane' tape was punched with a ¼-in. circular paper-punch and then placed in contact with the epidermis. The tape was peeled from the mesophyll tissue carrying an intact epidermal layer covering the punched-out area. The tissues were allowed to dry in the normal laboratory atmosphere of about 20–30 per cent humidity and were analysed 24–48 hr. later with a Perkin Elmer model 21 infra-red spectrophotometer. The spectrum of a normal lower epidermis from a cucumber cotyledon plotted against air in the reference beam is shown in Fig. 1.

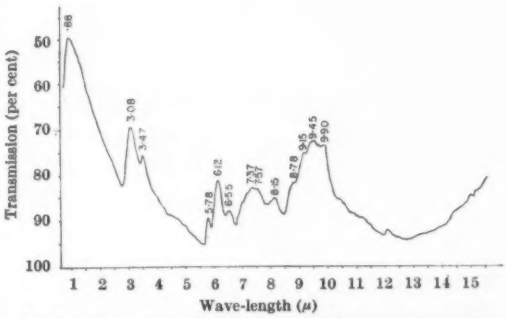

Fig. 1. Infra-red transmission through dried, normal cucumber cotyledon lower epidermis. Wave-lengths of principal peaks are identified

The analytical method used for detecting virus-induced changes was differential double-beam analysis with normal epidermal specimens in the reference beam and virus-infected epidermal specimens in the sample beam. The instrument then plotted the difference between the infected and normal specimens at all wave-lengths from 1 to 15μ. The recorder pen was adjusted to near mid-scale on the chart paper so that induced excesses and deficiencies could both be recorded as deviations from the I_0 (base line) of the instrument. These deviations were plotted linearly from I_0 in terms of percentage of the transmission of the normal tissue. Each of the infected tissues was compared with each of the normal tissues collected at the same time. The recorded spectral curves of each day's analyses were superimposed to give maximum coincidence of points. Median values were drawn for the superimposed curves and were plotted as values representative of the daily virus-induced changes. The daily median values plotted from two successive experiments were remarkably similar, indicating that the analytical method was reproducible. The results from both experiments are combined in Fig. 2 to show the progressive changes induced by the virus infection in the tissues as detected by the methods used.

Interpretation of the results presented in Fig. 2 is complicated by the numerous cellular reactions occurring within epidermal cells during the infection. Synthesis of virus nucleic acid and protein is probably accompanied by degradative reactions. Translocation of carbohydrates and possibly amino-acids, purines and pyrimidines to the halophytic epidermal cells may be altered by the changed rates of utilization of these compounds in cells infected by virus. Virus formation and side reactions may produce accumulated intermediate or end products that are not used in producing virus. The spectrophotometric changes

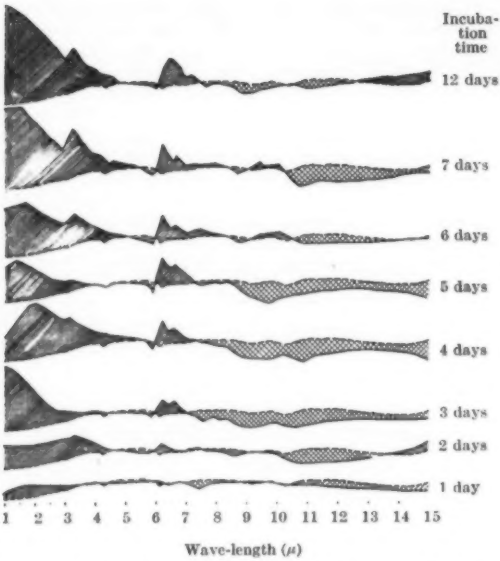

Fig. 2. Progressive changes in infra-red absorption of lower epidermis of cucumber cotyledons induced by the cucumber mosaic virus infection process (averaged values of two experiments). Full line, graphically determined median value for comparisons of three virus-infected with three normal epidermal strips; broken line, I_0 (base-line of the instrument); dark shading, greater than normal absorption; light shading, less than normal absorption

observed during the course of an infection represent the resultant effects that all the above processes have in adding to or decreasing the total number of chemical bonds that are responsible for absorption at each wave-length.

Increased spectral absorption in the infected tissues reflects an increased accumulation of certain chemical bonds that may be the result of increased translocation or of synthesis. A decreased spectral absorption in the infected tissues reflects losses of certain chemical bonds probably as a result of degradation reactions or perhaps by translocation.

The peptide linkage is known to give rise to strong absorption bands at 6·1 and 6·5μ (ref. 1). Because increased absorption at 6·5μ did not occur until the third day, it appears that a significant increase in peptide linkages did not occur until this time. The absorption at this wave-length continued to increase on the fourth and fifth days, but fell to a lower level on the sixth and seventh days. It is probable that this increase in peptide linkages reflects a comparable increase in proteins.

Nucleic acids and proteins both absorb strongly in the 3- and 6-μ ranges. It is possible that the absorption seen at these wave-lengths on the second day is caused by increases in nucleic acids. Nucleic acids also absorb strongly in the 9–9·5μ range[2]. On the second day there is a slight excess in this spectral region. On the third, fourth and fifth days marked deficiencies occur in this range and then on the sixth and seventh days excessive absorption occurs again. It is interesting to note that the absorption in the 9–9·5μ region appears to vary inversely with the absorption at 6·5μ. The results suggest that a cyclic variation has occurred in which nucleic acids build up and then fall during a period in which proteins are increased. Then when protein-levels fall on the sixth and seventh days nucleic acid-levels rise.

The relation of nucleic acid to protein synthesis is not well understood. Perhaps a comparison with studies of nucleic acid synthesis in virus-infected bacterial cells is noteworthy. An exponential increase of protein, ribonucleic acid and deoxyribonucleic acid occurs in a logarithmically growing population of bacterial cells. However, at the time of the inoculation of *E. coli* with T_2 bacteriophage the synthesis of bacterial components stops quickly and the cellular machinery is devoted to the synthesis of viral protein and viral deoxyribonucleic acid[3]. Astrachan and Volkin[4] have shown with tracer studies that a burst of synthesis of ribonucleic acid occurs before the formation of virus proteins. It is postulated that the synthesis of ribonucleic acid is a necessary prerequisite for the formation of the proteins essential to virus formation.

The results in Fig. 2 suggest that other reactions are occurring, but no attempt will be made at the present time to postulate these. Experiments are now in progress in which this type of analysis is being applied to the study of the earliest detectable events in the infection process.

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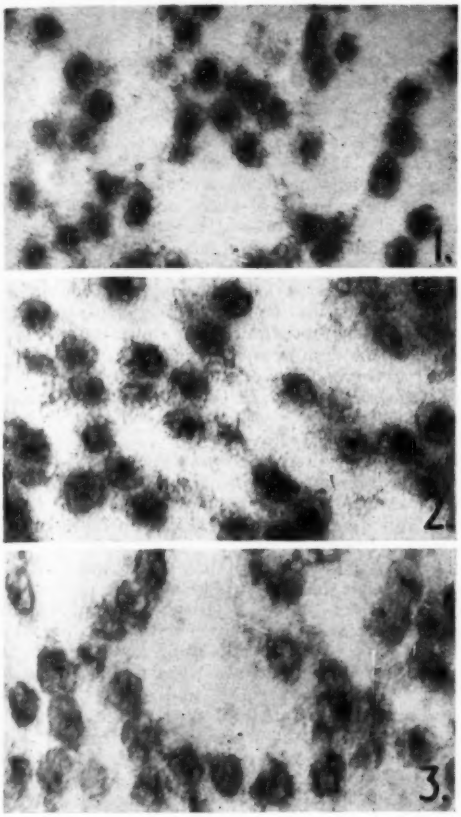
Site and Nature of Adenovirus Nucleic Acid

The effects of specific nuclease digestion on the morphology of the Rous virus, as observed with the electron microscope, established recently that this agent's nucleoid contained a substantial amount of ribonucleic acid[1,2]; the type of nucleic acid was confirmed at the same time by cytochemical studies[2].

Similar methods have now been used with type 5 adenovirus. The virus was grown in HeLa cell cultures[3], and particles were isolated from them by a fluorocarbon separation technique[4,5] followed by high-speed centrifugation. Samples of the particle-containing zones of the resulting pellets were used for the work and were first sectioned for electron microscopy in order to check their homogeneity[6]. Biological tests were combined with these experiments and served to indicate the viral nature of the particles[6].

Thereafter, other samples of pellets containing the virus were treated with nucleases following fixation with potassium permanganate[7], a fixative known to permit subsequent digestion of nucleic acids by such enzymes[2]. The nucleases were applied in many ways; but specific changes in virus morphology were only



Figs. 1–3. Electron micrographs of thin sections of permanganate-fixed adenovirus particles treated in various ways. All the particles were exposed to a preliminary incubation at 37° C. for 2 hr. in buffer at pH 9 containing 30 per cent alcohol, and show some degree of swelling as compared to examples prepared directly. × 75,000
Fig. 1. Control particles given a second incubation in 30 per cent alcohol containing 0·003 *M* magnesium chloride; the dense nucleoids are very evident
Fig. 2. Particles which had been exposed to ribonuclease at *p*H 7 (1 mgm. per ml.) during a second incubation in 30 per cent alcohol. No change has taken place in the density of the nucleoids
Fig. 3. Particles treated with deoxyribonuclease (1 mgm. per ml.) and 0·003 *M* magnesium chloride in 30 per cent alcohol during a second incubation. The nucleoids have been digested

observed after the following procedure. Samples of virus fixed in iced permanganate were placed in iced 30 per cent alcohol and brought to room temperature; they were then incubated for 2 hr. at 37° C. in Michaelis veronal buffer at *p*H 9 containing 30 per cent alcohol. After this the samples were divided into three groups and were incubated again in 30 per cent alcohol containing respectively 1 mgm. per ml. ribonuclease at *p*H 7, the same quantity of deoxyribonuclease together with magnesium chloride at a concentration of 0·003 *M* or, as a control, a similar concentration of the salt but no enzyme. At the end of this second incubation all the samples were dehydrated, embedded in *n*-butyl methacrylate and sectioned for electron microscopy.

Exposure to the alkaline buffer caused some degree of non-specific swelling of all particles as compared with examples prepared directly, while in the control preparations treated with enzyme-free medium after the buffer, no other structural change was seen (Fig. 1). A similar lack of change was found in particles treated with ribonuclease during the second incubation, the density of the nucleoids being well preserved (Fig. 2). In marked contrast, the nucleoids were digested from particles incubated with deoxyribonuclease after alkaline buffer (Fig. 3). The effect of the buffer was probably to degrade the outer coat of the particles in the manner already reported for mild alkalis[6,9], so allowing subsequent penetration by deoxyribonuclease.

These results demonstrate that the adenovirus contains much deoxyribonucleic acid, thus substantiating earlier inferential evidence[10], and also establish the location of this material in the nucleoid. A full account of this work will be given elsewhere[11] together with the confirmatory results of concomitant fluorescence microscopy tests for nucleic acids[12,13].

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ARCHÆOLOGY

The Enkomi Silver Cup

In *Nature* of May 28, p. 676, reference is made to a nielloed silver cup of 1400 B.C. from Enkomi, Cyprus. To encounter a nielloed article of such an early date would indeed be a discovery of importance, since, so far as we are aware, no one has proved the use of niello as a means of decoration before the second century A.D. (ref. 1). There is, however, no evidence for the presence of niello on this cup, as we were able to demonstrate at the time the cup was being restored. The paper dealing with this[2] shows clearly that the only basis the author had for assuming the powdery material surrounding the gold ornamentation to be niello was that it was black.

It will be apparent, therefore, that the Enkomi cup should be made available for further scientific study; but in the meantime we should like to point out that the only conclusion that can be arrived at from the evidence reported in the paper[2] is that the Enkomi silver cup was inlaid not with niello, but with copper. It is pertinent to remark that, when last seen by us, the handle of the cup still retained some of its copper inlay.

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British Museum (Natural History), London, S.W.7. July 4.

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That the Enkomi silver cup exhibits many fascinating points of technique no one will deny. The suggestion that the black is derived from an inlay of copper is interesting, especially coming from the Department of Mineralogy of the British Museum (Natural History), and it is clear that such a copper-inlay technique would be likely to facilitate manufacture; but, in the absence of the inlays, its presence would be difficult and might even be impossible to prove. The cap at the extremity of the 'wish-bone' handle, referred to above, was scraped and the freshly exposed surface appeared coppery, but whether due to inlay or to staining of the silver alloy (that is, by niello) was not determined. Dr. Claringbull, who examined scrapings by spectrograph, could detect silver, copper and gold only. It is admitted that to obtain a *representative* sample of the black material in question from the bowl, without causing damage, was found to be most difficult. A further examination by X-ray diffraction of a second tiny specimen that was submitted yielded nothing fresh.

The features that led me to consider the Enkomi cup had been decorated with niello may be summarized: black patterns lustrous or splendent and enamel-like; where damaged, brittle and in one case appearing as a charcoal-like powdery stain subsisting from antiquity in contact with massive copper chlorides and carbonates; where undamaged, forming a perfect tracery of shiny jet-black mass-and-line ornament in a groundwork of bright metallic silver; the perfection of line and lustre survived immersion for 20 min. in commercial formic acid (1 : 2 parts water, by vol.), at boiling temperature, without any sign of loss. The gold-foil patterns that had been applied to this black material were in no way deformed, as one might have expected them to be, had they been mounted on copper inlays that would certainly have become swollen by oxidation during 2,000 years of burial. The cavities cut in the silver to contain the black decoration, where undamaged, were filled flush with the surface contour and the black material did not appear to have increased in bulk. If the results of the X-ray crystallographic examination were considered to be in doubt because of the possible inadequacy of the sample, the above observations—difficult to explain otherwise—had to be taken as straw in the wind suggesting the use of niello.

The weak point in the argument for niello is that although chlorides were present in quantity in the incrustation, no trace of sulphides could be detected. For this reason I agree that the case must be taken to be not proven as regards the presence of stromeyerite and acanthite; and, having regard to the importance of the matter, I would agree further that a re-examination is desirable. I suggest, however, that as we have now a new theory, some preliminary work should be done on black lustrous copper minerals (as exemplified, for example, in Tang mirrors) to determine how far the black compounds can survive treatment with hot formic acid without losing lustre, shape or quality, before contemplating further operations on the cup itself.

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MISCELLANY

Cost of Scientific Periodical Publications

I am writing with reference to the paragraph under the above heading which appeared in *Nature* of April 9, p. 124.

The periodicals mentioned are not similar in matter per page, since some of them contain considerable amounts of what is classed as 'difficult' setting by printers, and this has an appreciable effect on production costs. While it may be broadly true to say that a reduction in price leads to increased circulation, the optimum circulation obviously depends on several other factors; for example, the number of people engaged in research in a particular field. The journal which has the backing of a scientific society is in an advantageous position in that it has a guaranteed circulation to members of that society, as well as a subsidy in most cases.

The figures quoted in the table are approximately correct for 'library subscription rates' of the Pergamon Press journals mentioned, although, on the basis of the volumes completed on 1959 subscriptions, the price of *Physics and Chemistry of Solids* is 3·7*d.* per page (not 4·1*d.*), and that of *Reactor Science* is 8·2*d.* per page (not 8·9*d.*). However, the table as published is not the whole story so far as Pergamon Press subscription prices are concerned. Pergamon Press, in order to ensure maximum dissemination and to encourage individual scientists to own their own journals, have a specially low subscription price for private individuals, and the effect of comparing these subscription rates against the journals published by other publishers listed in the table is as follows:

Title	Source	Publisher	Price per page
Tetrahedron	U.K.	Pergamon Press	0·7*d.*
Journal of Inorganic and Nuclear Chemistry	U.K.	Pergamon Press	0·9
Journal of the Chemical Society	U.K.	Chemical Society	1·0
Physics and Chemistry of Solids	U.K.	Pergamon Press	1·0
Chemical Reviews	U.S.A.	American Chemical Society	1·2
Journal of Soil Science	U.K.	Oxford University Press	1·3
Chemical Engineering Science	U.K.	Pergamon Press	1·4
Information and Control	U.S.A.	Academic Press	2·0
Philosophical Magazine	U.K.	Taylor and Francis	2·2
Biochemie	Holland		2·25
Fuel	U.K.	Butterworth	2·5
Journal of Electronics	U.K.	Taylor and Francis	2·8
Chemical Abstracts	U.S.A.	American Chemical Society	3·0
Reactor Science	U.K.	Pergamon Press	4·1

Since this note was written, an amicable and constructive meeting has taken place between representatives of the Pergamon Press and the Standing Conference of National and University Libraries and the Library Association; the latter, although they now have a better appreciation of the reason for the Pergamon Press price differential policy, nevertheless still object to the fact that libraries should have to pay the higher, multi-user price.

I. R. Maxwell

Pergamon Press, Ltd., Headington Hill Hall, Oxford.

FORTHCOMING EVENTS

Monday, September 19

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. D. E. Weimer: "Decorative and Electronic Applications of Gold Plating".

Tuesday, September 20

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 11 a.m.—Meeting on "The Chemist and Food Quality".

Wednesday, September 21—Friday, September 23

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (in the William Beveridge Hall, Senate House, and the Assembly Hall, Institute of Education, University of London), at 9.30 a.m. daily—Symposium on "High Temperature Resistance and Thermal Degradation of Polymers".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL (graduate, or equivalent qualification, with teaching, administrative and industrial experience) OF HACKNEY TECHNICAL COLLEGE, London, E.8—Education Officer (FE3a/T/2066/9), London County Council, County Hall, London, S.E.1 (September 23).

RESEARCH ASSISTANT (with experience of research work in physical chemistry or physics and preferably with a Ph.D. degree in one of these subjects) IN THE DEPARTMENT OF CHEMISTRY, to continue work on a programme, under the general direction of Dr. S. J. Gregg, on the reaction of gases with metals at elevated temperatures. The techniques include measurement of the sorption of gases and the use of the vacuum microbalance and of radioactive tracers; special attention is paid to the part played by the film of reaction product—The Secretary, The University, Exeter (September 24).

RESEARCH FELLOW (preferably with experience in rocket or satellite instrumentation) IN PHYSICS, for research on primary cosmic rays using instruments carried in artificial earth satellites—The Secretary and Registrar, The University, Southampton (September 28).

ASSISTANT ANALYST (science graduate with some training in biochemical methods), in an experimental cytology unit working mainly on neurobiological problems—The Professor of Pathology, School of Medicine, Leeds 1 (September 30).

BACTERIOLOGIST, Assistant Experimental Officer grade (with a pass degree with bacteriology as main or subsidiary subject, or equivalent qualification), in the Humber Laboratory, Kingston-upon-Hull, to assist in research on bacteriological aspects of handling, processing and preservation of fish and fish products, together with certain fundamental studies of marine microbiology—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G323/0A (September 30).

HEAD (with high academic and professional qualifications in mechanical or electrical engineering together with appropriate industrial, teaching and administrative experience) OF THE ENGINEERING DEPARTMENT—The Registrar, Flintshire Technical College, Connah's Quay, near Chester (September 30).

SENIOR RESEARCH FELLOW IN PHYSICS at the University of New England, Armidale, N.S.W., to take part in a research programme to study the action of very powerful beams of gyro-waves on the ionosphere—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (September 30).

RESEARCH FELLOW IN PHYSICS at the University of Tasmania, for work with a group which is active in the field of intensity variations and associated solar-terrestrial phenomena, using counting techniques developed for the I.G.Y.—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (September 30).

LECTURER IN PHARMACOLOGY in the Department of Pharmacology and Therapeutics—Joint Clerk to the University Court, Queen's College, Dundee (October 1).

VETERINARY SURGEON (preferably a recent graduate with some practical experience and an interest in the application of chemical methods to the investigation of animal diseases) in the Agricultural Research Council's Experimental Pathology Department, Institute of Animal Physiology, to assist the Clinical Pathologist—The Secretary, Institute of Animal Physiology, Babraham, Cambridge (October 1).

LECTURER IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, The University, Aberdeen (October 3).

SCIENTIFIC ASSISTANT, for the maintenance of physical instruments, including spectrophotometers and radiochemistry equipment—Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (October 8).

JUNIOR RESEARCH FELLOW or RESEARCH FELLOW IN THE DEPARTMENT OF BOTANY, University College, Ibadan, Nigeria, for research into the biology of the parasite Striga—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 10).

ASSISTANT LECTURER (with special qualifications in physical chemistry) IN PHYSICAL CHEMISTRY—The Registrar, The University, Manchester 13 (October 15).

LECTURER IN PHYSIOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 15).

PRINCIPAL (with high academic qualifications and appropriate educational and administrative experience)—R. H. Currell, F.C.A., Northern Polytechnic, Holloway, London, N.7 (October 15).

PROFESSOR AND HEAD (with academic qualifications and research or development experience appropriate to a postgraduate department studying gas turbines, jet propulsion engines and rockets, with an increasing emphasis on high temperature problems and nuclear power) OF THE DEPARTMENT OF AIRCRAFT PROPULSION—The Principal, The College of Aeronautics, Cranfield, Bletchley, Bucks (October 15).

RESEARCH CHEMIST or BIOCHEMIST (honours graduate in science or agricultural science) IN THE DAIRY RESEARCH SECTION, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, to take part in an expanded programme of research on dairy manufacture—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2 (October 15).

SENIOR LECTURER and LECTURER IN MATHEMATICS at the University of the Witwatersrand, Johannesburg—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 15).

SENIOR LECTURER (with an honours degree, and preferably a higher degree in electrical engineering with specialization in electronics) IN ELECTRICAL ENGINEERING at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, October 15).

SENIOR RESEARCH ASSISTANT, and EXPERIMENTAL OFFICERS (2), to work under the direction of Prof. G. Gee on the physical properties of high polymers—Prof. G. Gee, Department of Chemistry, The University of Manchester, Oxford Road, Manchester 13 (October 15).

CHAIR OF MEDICAL RADIOBIOLOGY in St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 20).

SENIOR NUFFIELD RESEARCH FELLOW (graduate in either medicine or science, and with several years research experience) IN THE DEPARTMENT OF MICROBIOLOGY—The Registrar, University of Adelaide, Adelaide, South Australia (October 20).

DIRECTOR (with a sound scientific training and with the ability to co-operate with scientific personnel, the administrators and the occupiers of the tussock and mountain lands) OF THE TUSSOCK GRASSLANDS AND MOUNTAIN LANDS INSTITUTE, Canterbury Agricultural College, Lincoln, New Zealand—Mr. H. G. Hunt, Registrar, Lincoln College, Christchurch, New Zealand (October 22).

LECTURER (with an honours degree in philosophy and experience in teaching and research) IN CHARGE OF THE TEACHING OF PHILOSOPHY at the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Kuala Lumpur and London, October 22).

CHAIR OF MATHEMATICS—The Registrar, The University, Hull (October 31).

FOUNDATION CHAIR OF NUCLEAR ENGINEERING in the University of New South Wales, Kensington, New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia, October 31).

SENIOR LECTURER IN PHYSICS at the Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 31).

SENIOR LECTURER or LECTURER (with an honours or higher degree in science or engineering and with teaching and research experience in the fields of the physics of solids, the mechanics of solids or solid state chemistry) IN ENGINEERING MATERIALS at the University of Melbourne, for duties including lectures and supervision of laboratory classes for second and fourth year engineering students who are required to study engineering materials on the basis of solid state physics and chemistry—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (November 14).

PROFESSOR AND HEAD OF THE DEPARTMENT OF AGRICULTURAL ECONOMICS AND PROVINCIAL AGRICULTURAL ECONOMIST—The Registrar, The University, Nottingham (December 1).

ASSISTANT PROFESSOR (preferably with an interest in anæsthesia) OF PHARMACOLOGY—Prof. J. G. Aldus, Department of Pharmacology, Dalhousie University, Halifax, N.S., Canada.

ASSISTANT PROFESSOR (with Ph.D. or M.B. degree) IN PHARMACOLOGY—Prof. M. F. Murnaghan, University of Ottawa, Ottawa, Ontario, Canada.

ASSISTANT PROFESSOR or ASSOCIATE PROFESSOR (PHYSIOLOGIST, with an M.D. or Ph.D. degree and capable of independent research) IN THE DEPARTMENT OF PHYSIOLOGY—Prof. M. Beznak, University of Ottawa, Ottawa, Canada.

CHAIR OF AGRICULTURE in the Faculty of Agriculture, University College of the West Indies, St. Augustine, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 20 Woburn Square, London, W.C.1.

GEOLOGIST (with a degree in geology, general experience in geology and geophysics and a knowledge of the occurrence of ground water) with the Government of Kenya, for duties including the location of sites for water boring, geological mapping with special reference to occurrence of ground water and general investigations connected with engineering problems—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.112/7/044/C1.

INTERNATIONAL WOOL SECRETARIAT RESEARCH FELLOW (with a degree in physics or physical chemistry, and with some research experience), to undertake work on the structure of protein fibres generally and wool in particular—Dr. F. Happey, Head of the Department of Textile Industries, Bradford Institute of Technology, Bradford.

LECTURER (with an honours degree in chemical engineering or corporate member of the Institution of Chemical Engineers, with experience in research and in the operation and supervision of full-scale chemical plant) IN CHEMICAL ENGINEERING—The Registrar, The University, Nottingham.

LECTURER (with a good honours degree and practical experience, with an interest in fluid motion and its technical applications) IN FLUID MECHANICS—Prof. C. M. White, Imperial College of Science and Technology, London, S.W.7.

LECTURER (with qualifications in mineralogy and petrology and an active interest in pre-cambrian rocks and ore deposits) IN GEOLOGY at the University of Khartoum—The Registrar, University of Khartoum, care of Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

MASTER TO TEACH PHYSICS: and a MASTER TO TEACH BIOLOGY—The Head Master, King's College School, Wimbledon, London, S.W.19.

PLANT PATHOLOGIST (with a good honours degree in a biological science, with postgraduate experience in research or advisory work in plant pathology) in Tanganyika, for research and specialist advisory services in plant pathology—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/8/08/C1.

PRINCIPAL (male, with appropriate qualifications and experience) OF THE DUDLEY AND STAFFORDSHIRE TECHNICAL COLLEGE—The Chief Education Officer, Education Offices, 3 St. James's Road, Dudley, Worcs.

RESEARCH ASSISTANT (with at least two years electronics experience) AT THE NUFFIELD RADIO-ASTRONOMY LABORATORIES, Jodrell Bank, for development and construction of low noise parametric amplifiers for use with a 250-ft. radio telescope—The Director, Nuffield Radio-Astronomy Laboratories, Jodrell Bank, near Macclesfield, Cheshire (October 10).

RESEARCH FELLOW (Ph.D. standard in chemistry or physics and with a sound knowledge of electronics), and a RESEARCH STUDENT (to read for a higher degree in chemistry), for research on new developments of nuclear magnetic resonance spectroscopy—The Secretary, Department of Chemistry, King's College, Strand, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research: Building Research Station. Factory Building Studies No. 6: Noise in Factories. By A. G. Aldersey-Williams. Pp. iv+26. (London: H.M. Stationery Office, 1960.) 3*s*. 6*d*. net. [306

Air Ministry: Meteorological Office. Averages of Earth Temperature for the British Isles. Pp. ii+159. (M.O. 665.) (London: H.M. Stationery Office, 1960.) 10*s*. net. [306

Department of Scientific and Industrial Research. Report of the Warren Spring Laboratory 1959: Report of the Warren Spring Laboratory Steering Committee for the period 1st July 1958 to 31st December 1959, and the Report of the Director of the Warren Spring Laboratory for 1959. Pp. iv+27+4 plates. (London: H.M. Stationery Office, 1960.) 3*s*. net. [306

National Union of Teachers. The Universities and the Teaching Profession. By Sir Charles Morris. Pp. 15. Willing the Means. By Sir Ronald Gould. Pp. 11. The Crucial Factor: The Supply and Training of Teachers, 1960–1970. By E. L. Russell. Pp. 10. (Addresses delivered at the Annual Conference of the National Union of Teachers at Blackpool.) (London: National Union of Teachers, 1960.) [17

Committee of the Privy Council for Agricultural Research. Report of the Agricultural Research Council for the year 1958–59. Pp. vii+213. (Cmnd. 1069.) (London: H.M. Stationery Office, 1960.) 9*s*. net. [17

Building Research Station Digest, No. 135 (June 1960): The Permanent Supplementary Artificial Lighting of Interiors (PSALI). Pp. 7. (London: H.M. Stationery Office, 1960.) 4*d*. [57

Chelsea College of Science and Technology. Prospectus, Session 1960–61. Pp. 35. (London: Chelsea College of Science and Technology, 1960.) [57

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The Diffusion of Knowledge. By Dr. Michael Grant. (Foundation Oration, 17 March 1960.) Pp. 15. (London: University of London Goldsmiths' College, 1960.) [57

Peace at the Last: a Survey of Terminal Care in the United Kingdom. By H. L. Glyn Hughes. (A Report to the Calouste Gulbenkian Foundation.) Pp. 64. (London: Calouste Gulbenkian Foundation, United Kingdom and British Commonwealth Branch, 1960.) 5*s*. [57

Institution of Electronics, Northern Division. Catalogue of the Fifteenth Annual Electronics and Instruments Exhibition and Convention, 1960, held in the College of Science and Technology, Manchester 1. Pp. xx+120+28. (Rochdale: W. Birtwistle, Honorary General Secretary, 78 Shaw Road, 1960.) [57

Other Countries

Durban Museum Novitates. Vol. 5, Part 19 (15th April 1960): A New Species of Pipistrelle Bat (*Chiroptera*: *Pipistrellus*) from South Israel. By David L. Harrison. Pp. 261–267 (2 plates). 1*s*. 6*d*. net. Vol. 5, Part 20 (30th April 1960): A New Race of the Flat-Headed Bat *Platymops Barbatogularis* Harrison from Kenya Colony, with observations on the Anatomy of the Gular Sac and Genitalia. By David L. Harrison and J. D. L. Fleetwood. Pp. 269–284 (5 plates). 3*s*. net. Vol. 6, Part 1 (30th April 1960): Geographical Variation in the South African Populations of the Golden-Breasted Bunting *Emberiza flaviventris* Stephens. By P. A. Clancey and J. M. Winterbottom. Pp. 1–11. 2*s*. net. (Durban: Durban Museum, 1960.) [276

Year-Book of the Institute of Nutrition, Budapest, 1959. Pp. vi+62. (Budapest: Institute of Nutrition, 1960.) [276

Union of South Africa: Department of Commerce and Industries—Division of Fisheries. Twenty-ninth Annual Report for the period 1st April, 1957, to 31st March, 1958. (Reprint from *Commerce and Industry*, November, 1959.) Pp. 160. (Pretoria: Government Printer, 1960.) [276

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Instituto de Zootecnia, Rio de Janeiro. Publicacao No. 25: Algumas Pesquisas da Associacao de Sinais na Pelagem Equina. Por Prof. Raul Briquet, Jr. Pp. 8. Publicacao No. 29: Introducao Geral ao Melhoramento Genetico das Populaxoes (com aplicacao aos problemas zootécnicos). Por Prof. Raul Briquet, Jr. Pp. 57. Série Monografias, No. 1: Genética da Pelagem do Cavalo. Por Prof. Raul Briquet, Jr. Pp. 117. (Rio de Janeiro: Instituto de Zootecnia, Ministeiro da Agricultura, 1959.) [306

Canada: Department of Mines and Technical Surveys. Publications of the Dominion Astrophysical Observatory, Victoria, B.C. Vol. 10. No. 26: Apsidal Motion in the Spectroscopic Binary H.R. 8800. By R. M. Petrie. Pp. 459–466. 25 cents. Vol. II, No. 4: The Inner Chromosphere of the K-Type Component of 31 Cygni as observed at the 1951 Eclipse. By K. O. Wright. Pp. 77–141. 25 cents. Vol. 11, No. 6: Shock Waves in the Atmosphere of the βCephei Star 8W Vulpeculae. By G. J. Odgers and R. S. Kushwaha. Pp. 185–200. 25 cents. Vol. 11, No. 7: The Automatic Guider of the Dominion Astrophysical Observatory. By P. E. Argyle. Pp. 201–208+plate 7. 25 cents. Vol. 11, No. 8: A study of the Wolf-Rayet Stars H.D. 192103 and H.D. 192163. By Anne N. Underhill. Pp. 209–234+plate 8. (Ottawa: Queen's Printer, 1960.) [306

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United States Department of the Interior: Fish and Wildlife Service. Statistical Digest No. 49: Fishery Statistics of the United States, 1958. By E. A. Power. Pp. ii+424. (Washington, D.C.: Government Printing Office, 1960.) 2 dollars. [57

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State of Illinois: Department of Registration and Education. Natural History Survey Division. Biological Notes No. 41: Hawks and Owls: Population Trends from Illinois Christmas Counts. By Richard R. Graber and Jack B. Mills. Pp. 24. Biological Notes, No. 42: Winter Foods of the Bobwhite in Southern Illinois. By Harlow B. Mills. Pp. 35. (Urbana, Ill.: State of Illinois, Department of Registration and Education, History Survey Division, 1960.) [57

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